# 1986 ANNUAL REPORT
# USDA-ARS

## BIOLOGICAL CONTROL OF WEEDS LABORATORY - EUROPE,

## ROME, ITALY



BIOLOGICAL CONTROL OF WEEDS LABORATORY PERSONNEL

| | |
|---|---|
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| Stephen L. Clement | Research Entomologist, Transferred-6/86 |
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| Claudine Vincenti | Administrative Clerk |
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| Rouhollah Sobhian | Research Entomologist, American Embassy, Vienna, Austria, Thessaloniki, Greece |
| Antonio Taricone | Part-time Maintenance Man |
| Carla Marangoni | PIT Summer Laboratory Assistant |
| Anna Rita Barbacci | PIT Administrative Clerk |

Cover photograph

By Massimo Cristofaro

Eustenopus hirtus
(Coleoptera: Curculionidae)

The larvae of this weevil feed in the half-grown seed heads of Yellow Starthistle, destroying the seeds. This weevil is being screened as a promising candidate for biological control of Centaurea solstitialis.

NOT FOR PUBLICATION
NOTICE

The results of this report are preliminary and should not be quoted or discussed in publications without permission of the responsible scientist. If there is need to refer to this work, please correspond with the scientist and include a copy of the pertinent portion of your manuscript. The work should be cited as a personal communication and not in the bibliography. This report has an extremely limited distribution and is intended only to provide a means of communication among scientists and to provide a historical record of our laboratory.

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# INTRODUCTION

The year 1986 was an active one for the USDA-ARS Biological Control of Weeds Laboratory at Rome (which includes Dr. Rouhollah Sobhian at Thessaloniki, Greece). While no new insects were released this year we were busy filling the pipeline for future releases.

This year we were occupied with testing, introducing insects into quarantine in the U.S. and surveying for populations of weeds and insects for future exploitation. In addition we put a lot of effort in collecting insects to augment populations already released. All this work was done, despite the loss of one of our scientists, Dr. Steve Clement, who returned to the United States in June.

Our major activities were:

Yellow Starthistle:

Eustenopus hirtus testing progress

Larinus curtus chosen as candidate, surveyed for populations and attempted mass rearing in a field cage

Surveyed Greek Islands of Kos and Rhodes for populations of plants and rosette feeders

Collected Chaetorellia hexachaeta for testing, Bangasternus orientalis and Urophora sirunaseva for release.

Leafy Spurge

Simyra dentinosa, tested.

Aphthona abdominalis, tested.

Chamaesphecia from Euphorbia virgata in Romania, emerged at Rome and determined to be crassicornis.

Aphthona czwalini collected for testing in quarantine

Collected Oberea erythrocephala, Bayeria capitigena, Aphthona flava, Aphthona cyparissiae to augment populations already released in the United States.

Diffuse knapweed

Bangasternus fausti - petitioned and introduced into quarantine at Albany.

Aceria centaurea located populations of this candidate eriophyid mite for study and collections.

Our research this year has laid the foundation for important introductions in 1987 and beyond.

Paul H. Dunn,

Location Leader.

# LEAFY SPURGE PROJECT

(P. PECORA, L. FORNASARI, M. CRISTOFARO, M. STAZI)

## SUMMARY

In 1986 the work on leafy spurge addressed both the screening studies of two natural enemies of Euphorbia esula "complex" and the massive collections of insects to be shipped in the U.S. either for further studies in quarantine or field releases.

(a) Simyra dentinosa Frr. (Lep.: Noctuidae). Neonate larvae of S. dentinosa were tested on 38 plant species or varieties in 10 families. Besides the control plant (E. seguierana), complete development occurred on four North American biotypes of leafy spurge (E. virgata "group") from Nebraska, Montana, Wyoming and Oregon, on five Euphorbia spp. of Euro-asiatic origin (E. cyparissiass, E. lucida, E. dendroides, E. helioscopia, E. peplus), and on E. maculata and E. spathulata, two species which are indigenous to North America. All the species on which S. dentinosa completed the larval development belong to the subgenus Esula, except for E. maculata which is the subgenus Chamaesyce. Plant species attacked by other Simyra spp. will be tested in 1987.

(b) Aphthona abdominalis Duft. (Col.: Chrysomelidae). This beetle was tested with 24 plant species or varieties. Complete development occurred on E. lucida, North American biotypes of leafy spurge from Nebraska, Montana and Wyoming and on the control (E. esula). Bionomical studies were also conducted.

(c) Chamaesphecia sp. (Lep.: Sesiidae). Because of the poor rate of emergence and the lack of fertile eggs, no testing was made with this clear-wing moth in 1986. Specimens of Chamaesphecia sp. emerged at the Rome Laboratory in 1986 from infested plants of the E. virgata "group" collected near the Danube delta in Romania in 1985, were identified as C. crassicornis Bertel by K. Spatenka, Vûk PS, Peckeg, Czechoslovakia.

Massive collections of Bayeria capitigena (900 galls), Dasineura capsulae (10,000 mature larvae), Oberea erythrocephala (418 adults), Aphthona flava (228 adults), A. cyparissiae (1,150 adults), and A. czwalinae (550 adults) were made in Italy and Eastern Austria from early May to mid-July. These collections were sent to Albany, California for clean up, species verification and eventual field release.

# SIMYRA DENTINOSA

(P. PECORA - M. CRISTOFARO)

Report prepared by M. Cristofaro

## INTRODUCTION

Simyra dentinosa Frr. (Lepidoptera: Noctuidae), a univoltine moth whose larvae feed in silk webs or tents formed on the apex of Euphorbia spp., occurs in southeastern Europe, Armenia and Central Asia (Heinicke, 1965); in southern Russia, Asia Minor, Palestine and southern Siberia (Spuler, 1908).

According to Thurner (1964), larvae of S. dentinosa were found on E. myrsinites in Macedonia. In 1977 Paul H. Dunn[1/] found the larvae of S. dentinosa feeding on Euphorbia virgata var. orientalis in Afghanistan and in Turkey and L. A. Andres[2/] discovered larvae feeding on Euphorbia sp. in Dushanbe, Tadzhikstan (Southern Russia). In 1978, a colony of this species was found feeding on Euphorbia seguieriana, near Seres (Greece), by A. Rizza and P. Pecora[1/]. In 1982 another colony on E. seguierana was discovered by R. Sobhian near Lake Volvi, in northern Greece.

In the Paleartic region, besides S. dentinosa, the following Simyra spp. are recognized:

a) S. nervosa Den. & Schiff., associated with plants of the genera Euphorbia, Rumex, Hieracium and Tithymalus (Seitz 1913; Spuler 1908; Forster & Wohlfarth 1954-77);

1/ USDA Biocontrol of Weeds Laboratory, Rome, Italy

2/ USDA ARS Biological Control of Weeds Laboratory, Albany, CA.

b) S. albovenosa Gze., associated with plants of the genera Typha, Arundo, Carex, Iris, Acetosella and Graminaceae (Forster & Wohlfarth, 1954-77; Spuler, 1908; Rungs, 1956);

c) S. splendida Stdgr., which occurs in Central Asia (Seitz, 1913); its host plant is unknown;

d) S. buettneri Hering occurs in marshlands in Northern Germany and Southern Russia (Seitz 1913).

The Nearctic species Simyra henrici (Grote) is associated with Typha, Polygonum, Salix, Zea mays, Triticum aestivum, Secale cereale, Phleum pratensis, Dactylis glomerata and Phalaris canariensis (Decker & Maddox,1971).

In 1982, R. Sobhian (USDA Biocontrol of Weeds Lab., Thessaloniki, Greece) made preliminary host specificity tests, exposing four North American biotypes of leafy spurge (Montana, Nebraska, Idaho and Canada) and Euphorbia pulcherrima (Poinsettia) to neonate larvae of S. dentinosa, hatched from eggs collected on E. seguieriana near Lake Volvi, east of Thessaloniki. On all U.S. leafy spurge biotypes and the control, the larvae grew and pupated. On poinsettia (E. pulcherrima), a highly valued ornamental, the larvae just nibbled and then died without molting. (Sobhian, 1983 USDA Biocontrol of Weeds Laboratory Annual Report).

These results led us to select, S. dentinosa as a candidate for the biocontrol of leafy spurge in North America. Studies on the bionomics and host specificity were carried out in 1986 at the USDA Rome laboratory are reported here.

## BIOLOGY

MATERIAL AND METHODS: Eggs of S. dentinosa collected on E. seguieriana by R. Sobhian near Lake Volvi (Greece) on April 9, 1986, and sent to the USDA Rome Laboratory. About 1500 eggs, laid on the lower surface of leaves, were placed in 128 ml. hatching containers and kept in the laboratory quarantine (greenhouse) under natural photoperiod (temp. 21 ± 6°C; RH 63 ±24%).

To determine the number of instars of S. dentinosa, 200 1st instar larvae were placed on 10 potted plants of E. seguierana with a camel hair brush. Once a week, until pupation commenced, a sample of 10 larvae were killed and preserved in 70% ETOH. The remaining larvae were left undisturbed to complete their development. Later, the head capsule width and the body length of these preserved larva were measured. In mid-June the pupae were taken from their silken cocoon and transferred in plastic containers (15x15x20cm), alternating a layer of pupae with a 1-cm layer of cornmeal. These containers were kept in a controlled temperature (15°C ±1°) until early November then moved to an outdoor insectary to wait for adult emergence.

RESULTS: Disk-like eggs, 300-350/leaf, were counted on four leaves. Freshly laid eggs were light yellow, turning black in 3-5 days. The pre-eclosion period was 15-18 days and 99% of the eggs were fertile.

S. dentinosa has six larval instars (Table 1). The larvae of S. dentinosa are hairy on dorsal and pleural parts: the 1st instars show yellow and black bands and are less hairy than the later instars. The 2nd instar is black, while the 3rd to 6th are dark brown with light brown inter-segmental bands. Gregarious behavior was noted from 1st to 4th instar. Groups of 7-10 1st instar larvae moved to the top of a branch and fed on buds and on tender leaves, where they spun a silken web "tent" and molted inside it every 5-6

days. The subsequent instars moved to another branch and feeding started again. The 5th and 6th instars showed a solitary behavior. The larvae completed their development in ca. 30 days, pupating in a yellowish silken cocoon made either among leaves and or on the container walls.

## LARVAL SURVIVAL TESTS

### MATERIALS AND METHODS

To determine the host range of S. dentinosa, two experiments were set up with neonate larvae coming from the same stock of eggs used for the bionomical studies. Thirty-seven test plants in 10 families plus the control plant (E. seguieriana, from the area where S. dentinosa occurs) were included in the trials.

In the first experiment (TEST A), a bouquet of each test plant was infested with one neonate larva, and was kept in a 500 cc. cardboard cup, with a paper towel to absorb moisture. One cup served as a replicate, and each test plant was replicated 10 times. To allow a good air exchange in the containers, a 5-cm diameter central hole was made on each plastic lid, and covered with organdy cloth. The bouquet was replaced twice per week, and the amount of feeding was measured in $mm^2$ by using a transparent plastic grid.

The second test (TEST B) was set up, using potted plants in transparent plastic tubes cages (20cm diam; 50cm height). Each potted plant represented one replicate, and received 20 neonate larvae of S. dentinosa. The test plants, as well as the control, were replicated twice. The larvae were left undisturbed and fresh plants were replaced as necessary. The larvae produced a silken web in which they fed and moulted. These webs were removed and preserved in 500cc cups. Later the head capsules contained in each web were measured and the number of instars which developed on the various test-plants was determined.

Both experiments started on April 23 and ended on June 20, 1986, and were conducted in the quarantine greenhouse (Temp. 21 ±6°C; RH=63 ±24%; natural day length).

B) RESULTS

S. dentinosa completed larval development only on plants of the genus on Euphorbia. Eleven of these plants were in the subgenus Esula and only one in the subgenus Chamaesyche (Table 2). The larvae nibbled on Euphorbia exigua, but died in the 1st instar. Two individuals reached 3rd instar on Helianthemum apenninum causing moderate damage. No damage was observed on E. pulcherrima. On the remaining 26 species no feeding was observed and the larvae died in 3-5 days without molting.

The larvae of S. dentinosa required a longer period to complete their development on Euphorbia seguieriana than those tested on U.S. biotypes of leafy spurge. This difference was probably because the plants of E. seguieriana received from Greece were not in top condition. The lack of

synchronization between the period of larval growth of S. dentinosa and the phenology of E. dendroides, E. helioscopia, E. spathulata and E. maculata, resulting in a paucity of suitable leaf tissue, was probably the major factor causing poor larval development on these test plants.

## CONCLUSIONS

The host specificity tests were conducted on plants closely related to leafy spurge and on plants of economic importance, i.e. poinsettia. The results of these experiments indicated that S. dentinosa can develop only on plants in the genus Euphorbia. The development on the native American species E. maculata and E. spathulata, does not necessarily indicate that these plants would be suitable in nature. To better evaluate the host relation of S. dentinosa on these two species, additional testing -- i.e. oviposition and oogenesis (laboratory test), host recognition (field test) - will be conducted in 1987. Furthermore, to get a more complete picture on the host range of S. dentinosa, plant species attacked by other Simyra spp. will be tested in the coming season.

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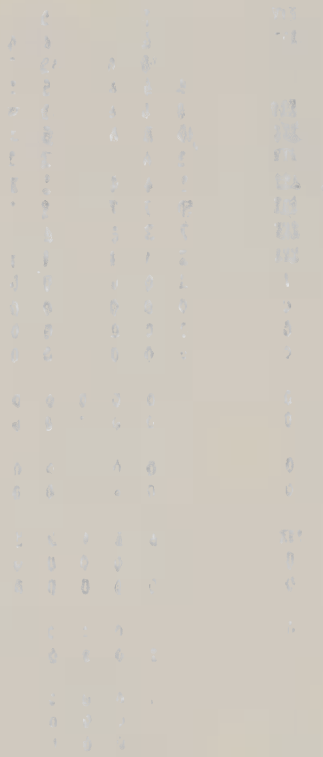
Table 1: Head capsule width of Simyra dentinosa larvae

| LARVAL STAGE | Head capsule width mm. $\bar{x} \pm$ SD (n= 20) |
|---|---|
| L1 | 0.41 ± 0.01 |
| L2 | 0.61 ± 0.03 |
| L3 | 0.90 ± 0.02 |
| L4 | 1.35 ± 0.04 |
| L5 | 2.28 ± 0.05 |
| L6 | 2.36 ± 0.02 |

TABLE 2: Plant species tested with first instars of Simyra dentinosa

| | TEST PLANTS | AMOUNT OF FEEDING 3/ | Test A 1/ NO. OF LARVAE SURVIVED TO THE FOLLOWING STADIA: II | III | IV | V | VI | P | Test A 1/ NO. OF DAYS ($\bar{X}$ + SD) TO COMPLETE LARVAL DEVELOPMENT | Test B 2/ NO. OF LARVAE SURVIVED TO THE PUPAL STAGE (%) |
|---|---|---|---|---|---|---|---|---|---|---|
| EUPHORBIACEAE | | | | | | | | | | |
| Subgenus Esula | Euphorbia seguieriana Neck. | XXX | 8 | 5 | 5 | 5 | 5 | 5 | 39.40 ± 1.57 | 11 (45.00) |
| | E.virgata(NEBRASKA) | XXX | 9 | 6 | 6 | 6 | 6 | 6 | 32.16 ± 0.75 | 12 (30.00) |
| | E.virgata(MONTANA) | XXX | 8 | 6 | 6 | 6 | 5 | 5 | 31.40 ± 1.34 | 20 (50.00) |
| | E.virgata(WYOMING) | XXX | 8 | 6 | 6 | 5 | 5 | 5 | 32.00 ± 1.08 | 13 (32.50) |
| | E.virgata(OREGON) | XXX | 8 | 6 | 6 | 5 | 5 | 5 | 31.50 ± 1.00 | 15 (37.50) |
| | E.cyparissias L. | XXX | 10 | 8 | 8 | 7 | 5 | 5 | 31.00 ± 0.70 | 14 (35.00) |
| | E.lucida W. et K. | XXX | 8 | 6 | 6 | 4 | 3 | 3 | 38.33 ± 2.13 | 10 (25.00) |
| | E.dendroides L. | XXX | 5 | 4 | 4 | 4 | 3 | 3 | 45.38 ± 3.03 | 5 (12.50) |
| | E.helioscopia L. | XXX | 10 | 7 | 7 | 4 | 3 | 3 | 54.13 ± 4.61 | 3 ( 7.50) |
| | E.peplus L. | XXX | 7 | 3 | 2 | 2 | 2 | 2 | 35.50 ± 4.94 | 5 (12.50) |
| | E.spathulata Lam. | XXX | 3 | 1 | 1 | 1 | 1 | 1 | 45 | 2 ( 5.00) |
| | E.exigua L. | X | 3 | 0 | 0 | 0 | 0 | 0 | | |
| | E.lathyris L. | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | |
| | E.characias L. | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | |
| | E.rigida Bieb. | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | |
| Subgenus Agaloma | E.marginata Pursh. | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | |
| | E.anthisyphilitica Zuccar. | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | |
| Subgenus Poinsettia | E.pulcherrima Willd. | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | |
| | E.heterophylla L. | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | |
| Subgenus Chamaesyche | E.maculata L. | XXX | 8 | 6 | 6 | 4 | 3 | 3 | 47.33 ± 4.04 | 3 (7.50) |
| | E.supina Raf. | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | |
| | E.serpyllifolia Pers. | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | |
| Subgenus Euphorbium | E.milii Ch. des Moulins | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | |
| | E.tirucalli L. | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | |
| | Mercurialis annua L. | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | |
| | Ricinus communis L. | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | |
| | Codieaum variegatum Blume | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | |
| CISTACEAE | | | | | | | | | | |
| | Helianthemum apenninum L. | XX | 4 | 2 | 0 | 0 | 0 | 0 | | |
| GERANIACEAE | | | | | | | | | | |
| | Geranium rotundifolium L. | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | |
| | Pelargonium zonale Ait. | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | |
| COMPOSITAE | | | | | | | | | | |
| | Cynara scolymus L. | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | |
| | Lactuca sativa L. | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | |
| LABIATE | | | | | | | | | | |
| | Salvia splendens Ker. Gavl. | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | |
| LEGUMINOSAE | | | | | | | | | | |
| | Medicago sativa L. | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | |
| RUTACEAE | | | | | | | | | | |
| | Ruta graveolens L. | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | |
| CONVOLVULACEAE | | | | | | | | | | |
| | Ipomoea grandiflora Roxb. | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | |
| SCROPHULARIACEAE | | | | | | | | | | |
| | Linaria vulgaris Mill. | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | |
| LINACEAE | | | | | | | | | | |
| | Linum usitatissimum L. | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | |

1/ TEST A : 10 neonate larvae (one/cup); a cup served as a replicate;
2/ TEST B : 20 neonate larvae / potted plant; 2 replicates/test plant;
3/ Amount of feeding estimated as: 0 = no feeding
X = nibbling
XX = moderate
XXX = heavy



# Leafy spurge (*Euphorbia esula* "complex")

## *Aphthona abdominalis* Duftschm.(Coleoptera: Chrysomelidae)

L. Fornasari and M. Stazi

## INTRODUCTION

Leafy spurge (*Euphorbia esula* L. "complex"), a group of plants of Euro-asiatic origin, are noxious weeds in north-central U.S. and are present in 26 States from coast to coast (Dunn, 1979), infesting ca. one million hectares in the U.S. and Canada (Alley and Messersmith, 1985). Because of its toxicity to livestock and the difficulty in achieving a long-term control (Lacey et al.,1985), leafy spurge is a serious problem. Chemical control is very often too expensive, therefore, biological control acquires considerable importance and an integrated control program, with biological, chemical and cultural means may be necessary (Lacey et al., 1985). Using biological control, several organisms attacking the various parts of the plant will probably be necessary to stress this weed sufficiently to abate it (Lacey et al., 1985).

The flea beetle *A. abdominalis* Duftschm. (Coleoptera: Chrysomelidae, Halticinae) is one of the candidate biological control agents of the complex of natural enemies of leafy spurge occurring in Europe which could probably be established in the U.S.

The CIBC Laboratory at Delémont, Switzerland has already screened and released several species of *Aphthona* for leafy spurge control. *Aphthona flava* and *A. cyparissiae* were released in Canada in 1982, *A. nigriscutis* was released in Canada in 1983. In 1985 *A. flava* was released in the U.S.A., and *A. czwalinae* was released in Canada.

Most of the flea beetles in the genus *Aphthona* Chevrolat are confined to host plants in the genus *Euphorbia* (Sommer & Maw, 1982).

The literature indicates A. abdominalis occurs on flax in Kazakhstan (Lakhmanov, 1970). We feel this record is the result of a misidentification and are trying to clarify it by testing A. abdominalis on flax at the Rome laboratory, and we have requested museum specimens from Lakhmanov (Soviet Union) for taxonomic determination .

The range of distribution of A. abdominalis includes northern and central Italy (Porta, 1934), Spain, France (Heikertinger and Csiki, 1939; Doguet and Tempère, 1975), Austria, eastern Yugoslavia, Balkans, Naxos, Hungary, Romania, Bulgaria, southern Poland, southern Soviet Union, Asia Minor and Iran (Heikertinger and Csiki, 1939; Heikertinger, 1944; Müller, 1949).

Pecora and Cristofaro first studied this insect in 1984 (USDA-ARS BCWL-E, Rome Annual Report). In 1986 studies on bionomics and host specificity were resumed,with the following objectives:

a) To complete the adult host range study started in 1984 (No choice feeding test);

b) To assess the ability of neonate larvae of A. abdominalis to develop on test-plants on which adult feeding occurred;

c) To verify if, under semi-natural conditions, feral adults are able to feed, oviposit and develop on various plant species;

d) To carry out observations and tests on the insect's development, adult longevity, fertility, and ovipositional behavior.

A) LIFE HISTORY

MATERIALS AND METHODS

EGG

The size, pre-eclosion period and the degree of fertility were determinated on eggs of A. abdominalis, laid by adults reared on E. esula in the laboratory garden. The eggs were kept in a constant temperature cabinet at 15° and 25° C ± 0.5° C, with 85-95% R.H., D:D photophase; ambient temperature and R.H., recorded. Groups of 20 newly oviposited eggs were placed in 35 ml. plastic cups provided with a layer of moistened plaster of Paris on the bottom. Each cup represented a replicate. Six hundred eggs=(30 replicates) were tested at each temperature. The number of hatched or collapsed eggs was recorded daily. Testing began early May and finished mid-August 1986.

LARVA

Preliminary observations were conducted on the larval behavior of A. abdominalis on E. esula using a colony of this flea beetle established on leafy spurge plants in the Rome laboratory garden. The aerial and terrestrial parts of 10 potted plants of E. esula were carefully examined on May 9 and on July 22. In addition laboratory trials were also made in September, by placing neonate larvae of A. abdominalis on root shoots, root buds and pieces of roots of leafy spurge. Thirty neonate larvae (1 larva per piece of plant part) were tested for each plant part, equally distributed in glass vials, gelatin capsules, and Petri dishes provided with moist blotting paper on the bottom, and kept at constant temperature (25±1°C). Plant parts were dissected at 4-6 day intervals and living larvae were transferred to fresh plant pieces.

ADULT

Biological data - i.e. preoviposition and oviposition periods, egg production, number of days of survival - were obtained on 100 adults of A. abdominalis collected on leafy spurge plants in the laboratory garden on April 4, 1986. These adults were equally distributed in 50 cylindrical plexiglass cages (height = 10 cm.; diameter = 7 cm.) whose tops were tightly covered with a fine mesh nylon net, with vials of leafy spurge bouquets introduced through holes at the bottom. Every 3-4 days, bouquets were replaced with fresh ones, eggs were collected and counted, and the number of living and dead individuals was also recorded. Since it was difficult to distinguish the sex of living adults using external morphological characters, their sex was determined by dissecting them once they died. Observations were conducted on these adults throughout their life span until they died in autumn. This trial, which started on April 4 and ended on September 9, was conducted in an outdoor insectary where temperature and humidity were recorded.

From the end of March to the beginning of December 1986, inspections at regular intervals were made in the laboratory garden on E. esula (from S. Rossore, Italy) and on the American biotypes of leafy spurge (from Montana, Nebraska, Wisconsin and Wyoming) recording preliminary data on the population density of A. abdominalis adults throughout its period of activity. Every 7-10 days the number of adults seen on the aerial portion of a sample of 150 plants was recorded.

The oviposition behavior was investigated on potted plants. On July 29, ten pots (10 cm. diameter) with E. esula plants from S.Rossore were caged with 10 ovipositing A. abdominalis each (15-20 days old). On August 7, 1986 they

were inspected and the trial terminated. Observations on feeding behavior were made on groups of 10 insects living on larger plants (in 22 cm. diameter pots) caged in transparent plastic tube cages (60 high x 20 cm.dia.).

To observe the adult oviposition and feeding behavior in more detail, three E. esula plants (S. Rossore) were put into special cages constructed for this purpose with the same principle used by Müller (1988). The roots with soil and the lower part of the stem were sandwiched between two glass panes (16x31 cm) with a wooden frame inside to separate the glass and provide space for the soil and roots, Fig.1. The plant stem passed through a hole at the top of the frame leaving the aerial portion outside the cage. Ten ovipositing A. abdominalis adults (15-20 days old) were put in each cage and observed daily, at regular intervals throughout the whole day. Ten days later the cages were opened to look for the oviposition sites and the eggs found were recorded. In a second trial the cages were modified and made with a polycarbonate plastic "Lexan" (General Electric Plastics Europa). This material has a completely smooth surface, with no crevices where the small larvae can hide and it is transparent, odorless and break resistant.

Further observations on a population of 642 adults collected in the garden at the beginning of October are continuing.

## RESULTS

### EGG

Just laid eggs are glossy white to light yellow, partially hyaline and with a tender chorion (see Fig. 2). The body of the larva is visible through the chorion of mature eggs a few hours before hatching.

The length of eggs (n=30) was: $\bar{x}$ 0.517±0.029 mm.; width was $\bar{x}$ 0.253±0.022 mm. The results of the egg eclosion tests carried out in temperature cabinets and in the garden are summarized in Table 1.

At 15° C it took $\bar{x}$ 33.2 days for eggs to hatch, at 25°C, it took $\bar{x}$ 8.7 days (with a small standard deviation). Eggs at outdoor temperatures (see figure 4) required $\bar{x}$ 13.1 days to hatch. Figure 3 shows the number of neonate larvae which emerged daily under different temperature regimes.

LARVA

In the laboratory, feeding tests with first instar larvae on different parts of the plant, suggest that they feed on underground root buds, but not on roots themselves.

Five days after the beginning of the experiment, live larvae were found in the inner part of small adventitious stem buds. The best technique to maintain the buds was to put them in glass vials closed with "parafilm M" and keep them in the dark; this system keeps the buds humid and in good shape and not too moist.

The same kind of damage on adventitious subterannean stem buds was found on garden grown leafy spurge plants heavily infested by A. abdominalis. In the ten plants dissected, about 50 buds were attacked. Buds damaged by A. abdominalis larvae are shown on figure 6.

ADULT

A. abdominalis adults average 2.0±0.1 mm. long (n=30) range 1.8-2.2 mm. and 1.0±0.1mm. wide (n=30) range 0.8-1.2. The adult has a reddish-yellow head and prothorax, black mesothorax, metathorax and abdomen. Elytra and leg teguments are light, bright yellow.

The average pre-oviposition period of these adults (n=35) was 30.5±22.6 days. During the whole oviposition period, females (n=38) laid $\bar{x}$ 24.9 ± 27.2 eggs each (range 1-104 eggs). The eggs were laid during a period of $\bar{x}$ 25.8 ± 31.0 days. The great difference in the number of eggs laid/female was largely due to the different oviposition period which ranged between 1 and 113 days. Females (n=38) were longer-lived, living for $\bar{x}$ 86.3±32.8 days (range 35 to 164 days). The longevity of the males (n=38) was $\bar{x}$ 64.3±27.9 days (range 24 to 154 days). The life span of males and females is shown in figure 8. A few matings were observed in the course of A. abdominalis life history study.

On March 28, 1986, A. abdominalis adults were first found in the laboratory garden on potted E. esula plants brought from Pisa in 1983. Their number increased quite rapidly and a week later 68 adults were seen on the 150 plants examined ($\bar{x}$ 0.43 adults per plant), but in the same period no adults were found on the American biotypes of leafy spurge (from Nebraska, Montana, Wyoming and Wisconsin) in the garden. Ten days later, on April 16, the first A. abdominalis adults were found on the American biotypes (seven adults on a sample of 150 plants). The population density of A. abdominalis was almost stable on both Italian and American biotypes until the end of June. At the beginning of July the A. abdominalis population increased dramatically (probably a new generation) to 155 adults on 150 leafy spurge plants of both Italian and American origin. The same density was observed in August. In mid-September only twelve adults were found feeding on 150 plants of the American biotypes, while 53 adults/150 plants were counted on the Italian E. esula plants. From the end of September the number of adults on the plants started decreasing gradually and on November 17, 1986, only two adults were

found feeding (at 2 p.m.; air temperature 18°C, cloudy, no wind) on 900 Italian E. esula plants inspected. No adults were seen on the American biotypes (n = 150). On December 2, only one adult was found feeding (at 2:30 p.m.; air temperature = 14°C, sunny, no wind)on the E. esula of Italian origin. A. abdominalis evidently overwinters as an adult.

The investigations on the oviposition and feeding behavior, conducted on potted plants in the laboratory, on plants in glass cages and in the garden, led to the following results:

(1) During the night the A. abdominalis adults were motionless on the soil or on the plants. Early in the morning (4.00 to 7.00 A.M.) they were still motionless. During the day they walked and fed on leaves, particularly on the upper part of the plants. Movement by this insect was very limited when it was on its host plant. Mating was not very frequent.

(2) The A. abdominalis females laid eggs singly or in groups of 2-6 in the soil very close the plant or at 1-5 cm. below soil level where they were found both along the primary stem and on the young adventitious stem buds on the roots.

(3) An observation of the out-of-doors population on October 31 showed that by that time of year, the adults stopped laying eggs, reduced feeding and activity in general, even if the temperature conditions were good (25°C), with L:D=11:13 photoperiod. Forty of these adults, put into a temperature cabinet at 25°C and with long daylight conditions (L:D = 15:9), started to feed and lay eggs again. This behavior continued until mid-December, when they died.

(4) Six hundred and forty two adults collected in the garden at the beginning of October and kept in cages in the garden, stopped feeding by December 9, 1986 (temperature: min.= 5°C; max. = 17°C). At the end of December they were still alive and observations are continuing.

B) TESTING

MATERIALS AND METHODS

No choice feeding test (laboratory)

In 1986, to determine the host range of A. abdominalis, the following plants were used in a no-choice feeding test: Linum usitatissimum, Helianthenum apenninum, Euphorbia supina, and E. serpyllifolia. E. esula (Pisa) was used as the control. Small bouquets of these plants were put in 500 ml. paper cups, each containing five adults of A. abdominalis collected in the laboratory garden from E. esula. The bouquets were replaced twice weekly. The number of dead adults was recorded, and leaf area consumed on the old bouquet was estimated by putting a transparent plastic grid divided into $mm^2$ units over the leaves and counting $mm^2$ leaf area eaten. This experiment was conducted from June 17 to July 23 in a laboratory room, with natural light and ambient temperature.

First instar larval survival test (laboratory)

The objective of this trial was to determine the ability of neonate larvae of A. abdominalis to survive on test plants, on which adult feeding activity was observed in the no choice feeding tests made in 1984 and 1986.

The larvae used in this test came from eggs laid by adults collected in the laboratory garden and the kept in paper cups with E. esula bouquets. The eggs were kept into a temperature cabinet at constant temperature (25°C $\pm$ 0.5°C) on moist blotting paper in Petri dishes until eclosion (about 8 days). Five neonate larvae were put on the root collar of the plants at soil level with a fine paint-brush and two weeks later transparent plastic tube

cages were placed over the plants. The test plants were inspected daily for adult emergence. Five replications were made of each test plant and the number of newly emerged adults was recorded. The test plants included in this trial are listed in Table 2 and the time from infestation to emergence on the acceptable plants is shown in Table 4.

Multiple Choice Host Suitability Test (field)

The objective of this test was to verify if under seminatural conditions feral adults of A. abdominalis are able to oviposit, feed and complete their life cycle on some North American biotypes of leafy spurge, on species closely related to E. esula and plants of economic importance. The trials took place in July and August 1986 at Castelporziano (Rome), a natural preserve where A. abdominalis did not occur. No other Euphorbia spp. were present in the experimental area. The test plants, in 22-cm.terracotta pots, were buried with the tops of the pots at ground level and assembled in two plots. These plots were about 5 km. from each other and are considered as separate experiments.

In Plot 1 the following plants were present: E. marginata, E. maculata, E. trigona, E. milii, E. tirucalli, E. antisyphilitica, E. serpyllifolia, E. supina, E. spatulata, Ricinus communis, Linum usitatissimum, and E. esula from Pisa (Italy) as control.

In Plot 2, we tested only the American biotypes of leafy spurge, to compare the preference of A. abdominalis between these and E. esula of Italian origin, avoiding interference by other Euphorbiae species. The following plants were tested: leafy spurge biotypes from Montana, Nebraska and Wisconsin, and E. esula from Pisa as control.

The experimental design was a randomized complete block and each plot had ten blocks. The distance between plants was 80 cm, and the plants were put in the ground one week before starting the tests. The insects used in these

tests were collected in the laboratory garden on E. esula plants. Ten sexually active A. abdominalis adults were released on each plant (total 1,100 adults in the plot 1 and 400 in plot 2). Twelve days after the insects were released, those remaining on the plants were collected and recorded and the test plants were dug up and brought back to the laboratory. At the laboratory the adults were checked to see if they were still ovipositing. The potted plants were then caged in transparent plastic cylinder cages and kept in a greenhouse until new adults emerged, about a month later.

RESULTS

No-choice feeding test

The results of this test are summarized in Table 3 and in Figures 9 and 10. Feeding occurred on all the test plants except Linum usitatissimum. The amount of feeding differs greatly between test plants. Considerable feeding occurred only on E. supina and on the control. All the insects died within a month except those feeding on the E. esula control. All these adults but one were still alive at the end of the experiment (30 days). On E. supina daily consumption per insect was high ($\bar{x}$ 8.8 $\pm$ 1.67 $mm^2$) in comparison with E. esula, it was about 78% of the mean daily consumption in the control ($\bar{x}$ 11.3 $\pm$ 2.01 $mm^2$).

Total feeding was 1080 $mm^2$ for E. supina and 1620 for E. esula (Fig. 9). On E. serpyllifolia there was considerable feeding at the beginning of the trial, but the life span of the insects (n=5) was very short ($\bar{x}$ 5,8 $\pm$3.83 days) resulting in a low total feeding (135 $mm^2$), shown in Fig. 9. On H. apenninum only nibbling (3 $mm^2$ eaten) occurred and in a few days ($\bar{x}$ 6.4 $\pm$ 3.51) all the test insects were dead. In figure 10 it is also evident that feeding stopped, because all adults died.

In this test ambient temperature ranged between 20° and 27° C and R.H. between 60 and 80%.

Larval survival test

A. abdominalis larvae completed their development only on the control, the American biotypes of leafy spurge and E. lucida. The larval development in the various plants tested took about 30 days. The development times and the number of emerged adults are reported in table 4. The number of adults emerging from the various test plants is shown in figure 11.

Multiple choice host suitability test

Figure 13 provides the results of the first trial(plot 1), with different test plants. Feral adults of A. abdominalis were able to feed, oviposit and complete development on some species, but in low numbers. From the control 210 adults emerged, 18 adults emerged from E. supina, 7 from E. marginata, 3 from E. maculata and no adults emerged from Ricinus communis or Linum usitatissimum.

In the second field trial (plot 2) 206 adults emerged from the control, 150 from the Nebraska biotype, 66 from the Wisconsin biotype and 35 from the Montana biotype, showing that the American biotypes are acceptable as hosts. This information is shown graphically in Fig. 14.

The A. abdominalis adults used were still ovipositing at the end of the trial.

## CONCLUSION

A. abdominalis is a promising candidate for the biological control of leafy spurge. It is multivoltine, the adults feed heavily on leaves of the leafy spurge plants and larvae feed on stem buds on aventitious roots instead of roots. Both larvae and adults feed actively from April to October. In both field and laboratory experiments, the flea beetle completed development on Euphorbia spp. only, including the American biotypes of leafy spurge. Next year we plan to complete the larval survival test and life history studies.

Table 1. Egg development time at different temperatures.

| Temperature | Days to eclosion $\bar{X}\pm$S.D.(n=600) | Percent |
|---|---|---|
| 15°C | 33.2±6.51 | 57.1 |
| 25°C | 8.7±1.05 | 69.7 |
| Outdoors | 13.1±1.96 | 81.0 |

Table 2. Plant species used in the Larval survival test.

| FAMILY | SPECIES |
|---|---|
| Euphorbiaceae: | *Euphorbia esula from Pisa-Italy, as control |
| | *E. esula-virgata from Montana, U.S.A. |
| | *E. esula-virgata from Nebraska, U.S.A. |
| | *E. esula-virgata from Wyoming, U.S.A. |
| | E. trigona |
| | E. milii |
| | E. antisyphilitica |
| | E. tirucalli |
| | E. characias |
| | E. supina |
| | E. serpyllifolia |
| | *E. lucida |
| | E. peplus |
| | E. marginata |
| | E. lathyris |
| | Ricinus communis |
| | Codiaeum variegatum |
| Linaceae: | Linum usitatissimum |
| Cistaceae: | Helianthemum apenninum |
| Geraniaceae: | Pelargonium zonale |
| Lythraceae: | Lythrum salicaria |
| Moraceae: | Ficus elastica |
| Convolvulaceae: | Ipomoea grandiflora |

* = Plants on which larval development occurred (for details see Table 4).

Table 3. No-choice feeding test with A. abdominalis adults - 1986

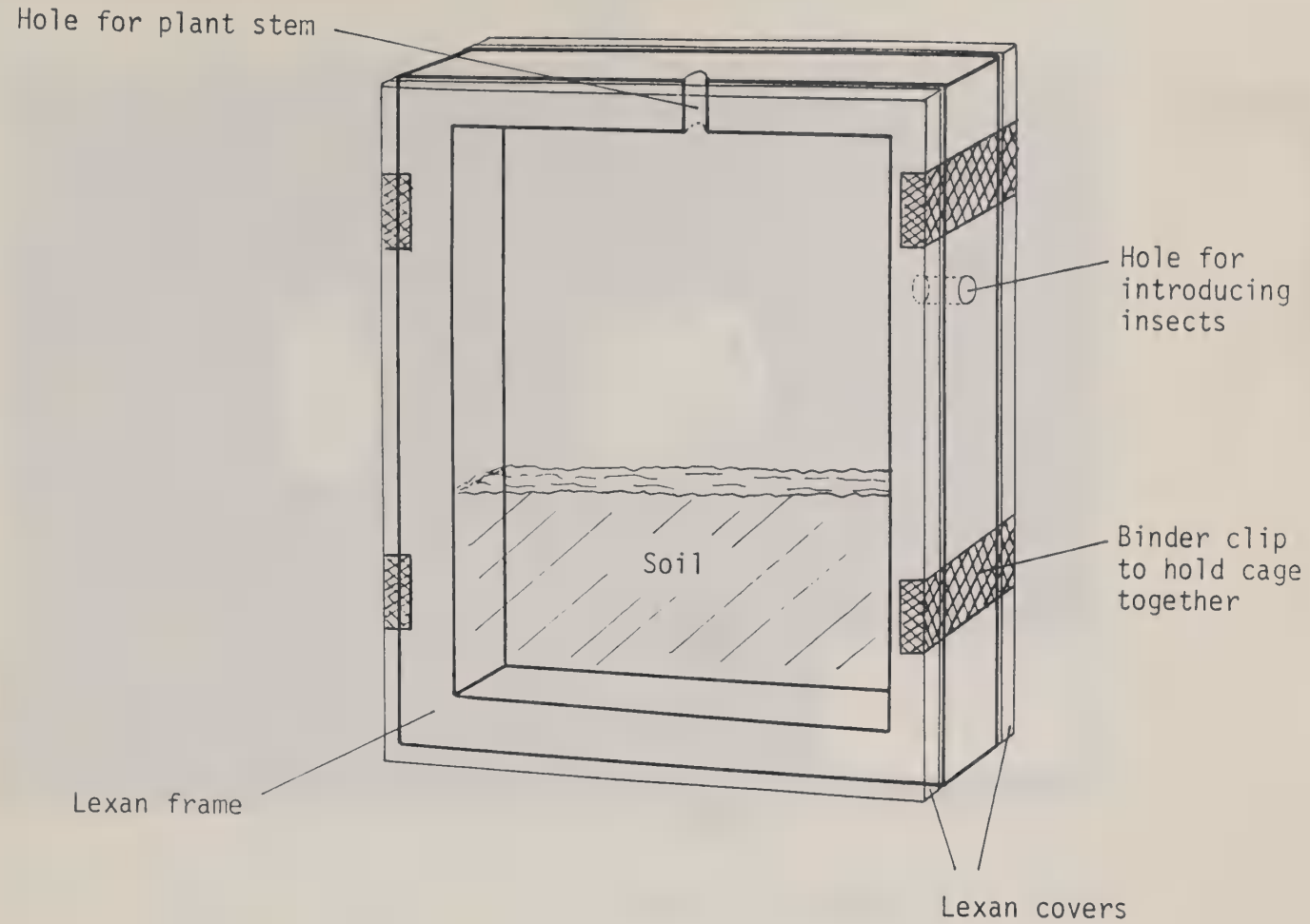
| PLANTS | $mm^2$ eaten/day per insect $\bar{x}$ ±SD | Feeding range ($mm^2$/day per insect) | % Feeding (1) | Survival (days) $\bar{x}$ ±SD |
|---|---|---|---|---|
| (Control) E. esula Pisa | 11.3±2.01 | 7.5 - 13.3 | 100 | 28.8 ± 0.45 (4 still alive) |
| Helianthenum app. | 0.15 ± 0.0 | - | 1.3 | 6.4±3.51 |
| E. supina | 8.8 ± 1.67 | 6.0 - 11.3 | 77.9 | 24.4±5.13 |
| E. serpyllifolia | 4.1 ± 2.65 | 2.25 - 6.0 | 36.3 | 5.8±3.83 |
| Linum usitat. | 0 | - | - | 3.8±1.09 |

(1) % feeding: $\frac{\text{mm2 eaten per insect per day on test plant}}{\text{mm2 eaten per insect per day on control}} \times 100$

Table 4. A.abdominalis first instar larva survival trial (1986)[1/]

| Test Plants | No. of adults emerged | % survival | Development time (days) Larva to adult ($\bar{x}$ = ± SD) |
|---|---|---|---|
| E. esula (control) | 18 | 72 | 32.5 ± 3.26(n =18) |
| E. esula (Montana ) | 4 | 16 | 32.0 ± 3.37(n = 4) |
| E. esula (Nebraska) | 4 | 16 | 29.7 ± 1.50(n = 4) |
| E. esula (Wyoming) | 1 | 4 | 33.0(n = 1) |
| E. lucida | 17 | 68 | 30.8 ± 4.10(n = 68) |

1/ 25 neonate larvae/test plant



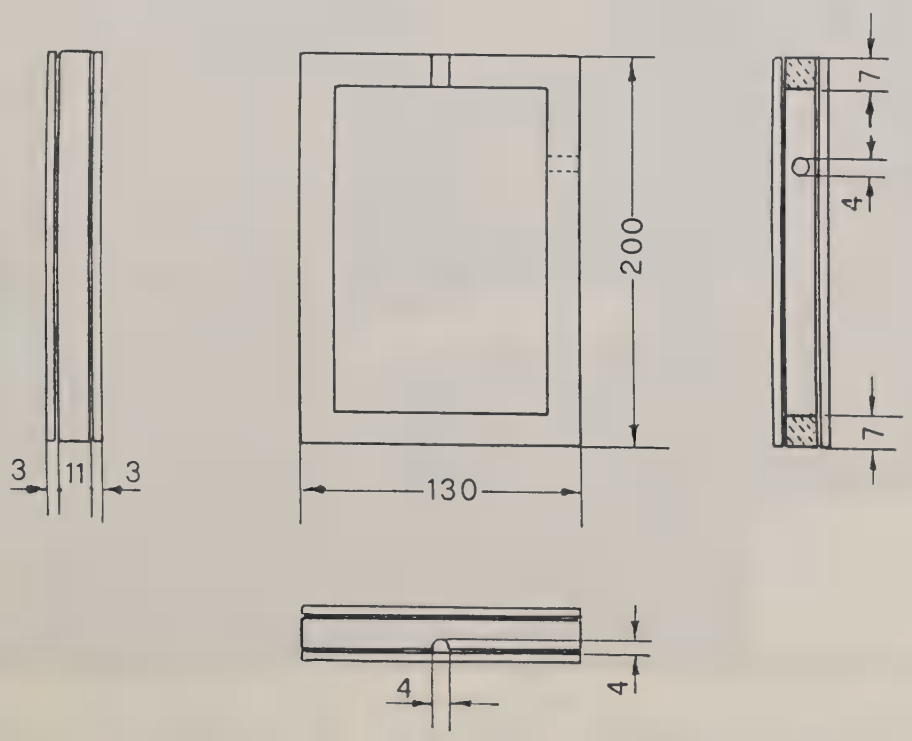




Fig. 1 : Lexan cages used for observations on A. abdominalis oviposition behavior (given dimensions are in mm.)

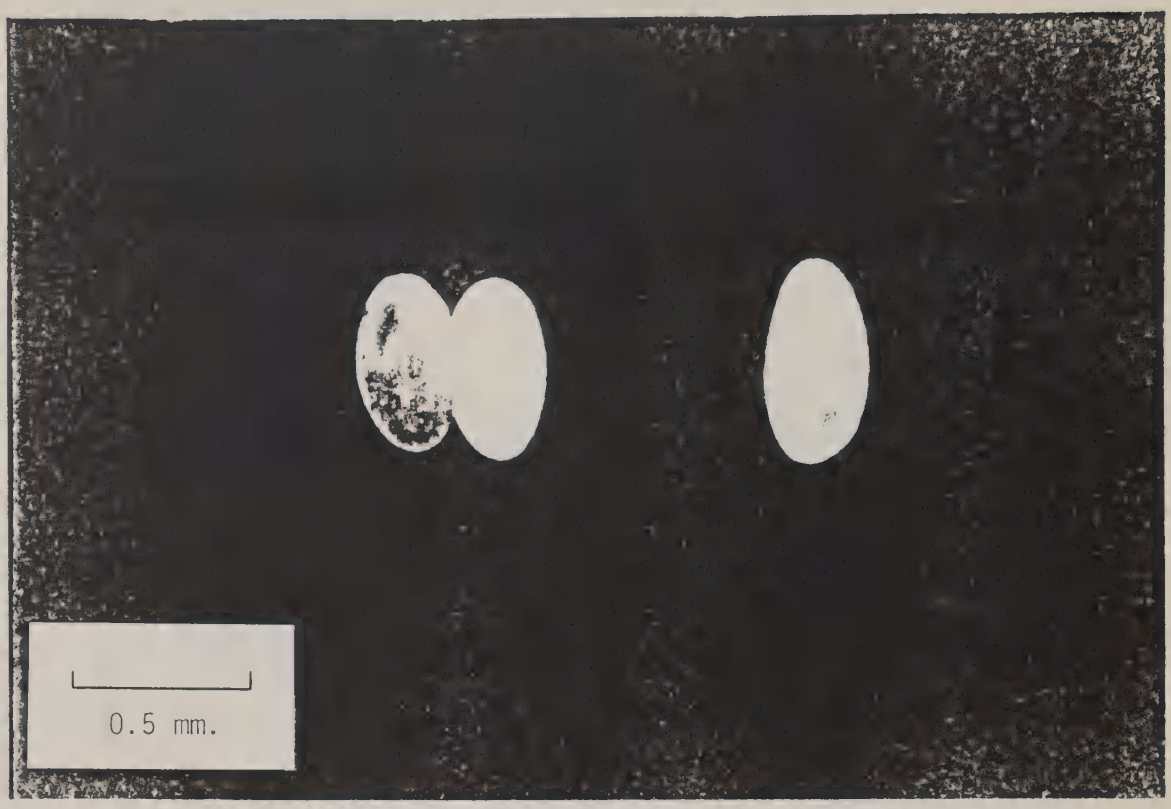



Fig. 2 : A. abdominalis eggs.


Fig. 5 : A. abdominalis first instar larva.

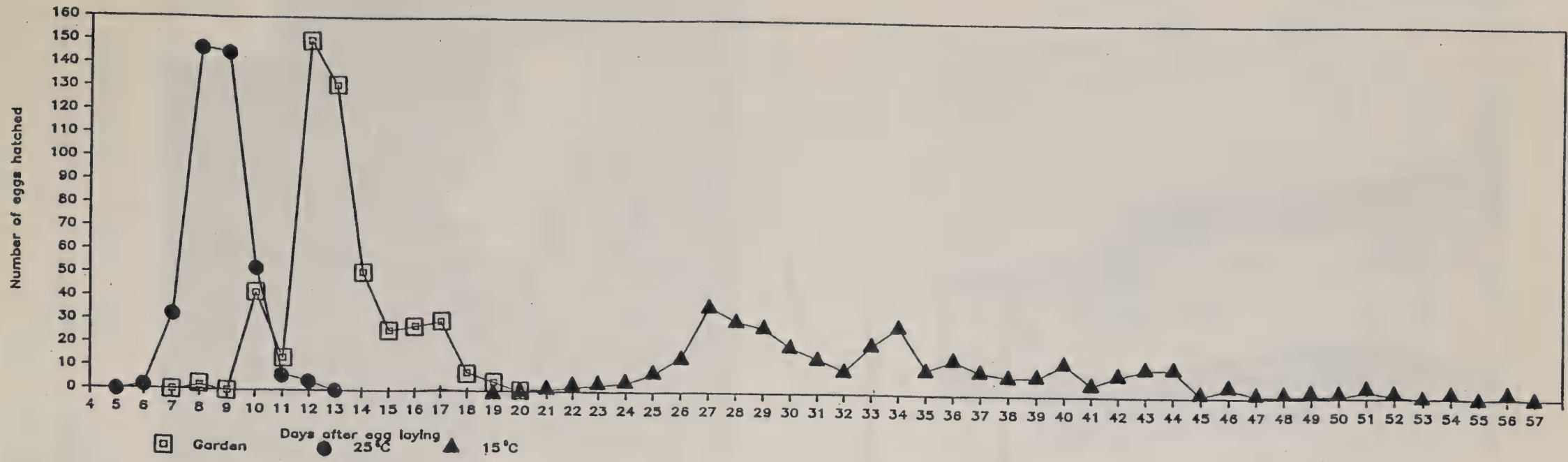



Fig. 3 : Egg hatching times at different temperature conditions.

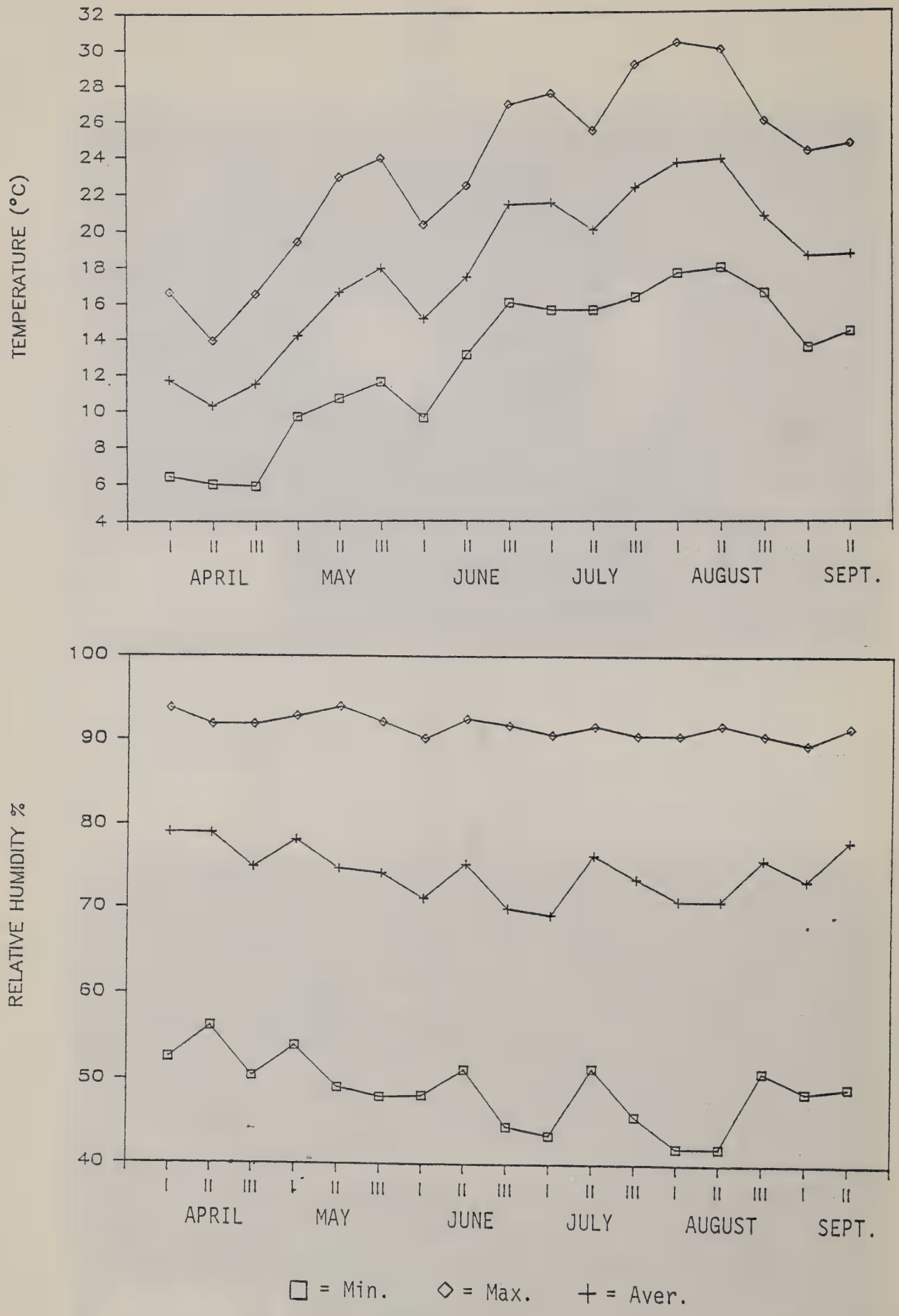



Fig. 4: Ten day means of tempearture and humidity in the garden, 1986.


Fig. 6 : Damage of A. abdominalis larvae on E. esula shoots.

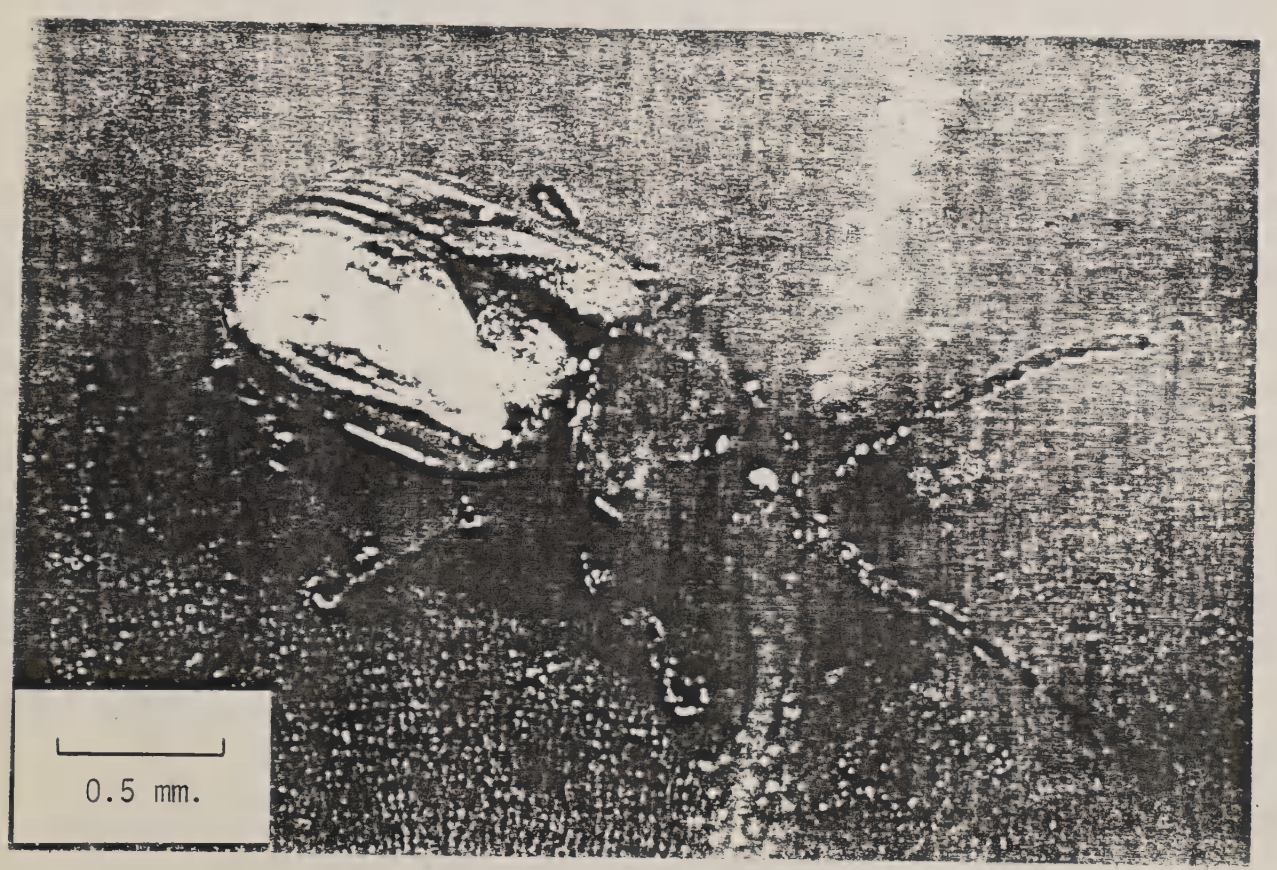



Fig. 7 : A. abdominalis adult.

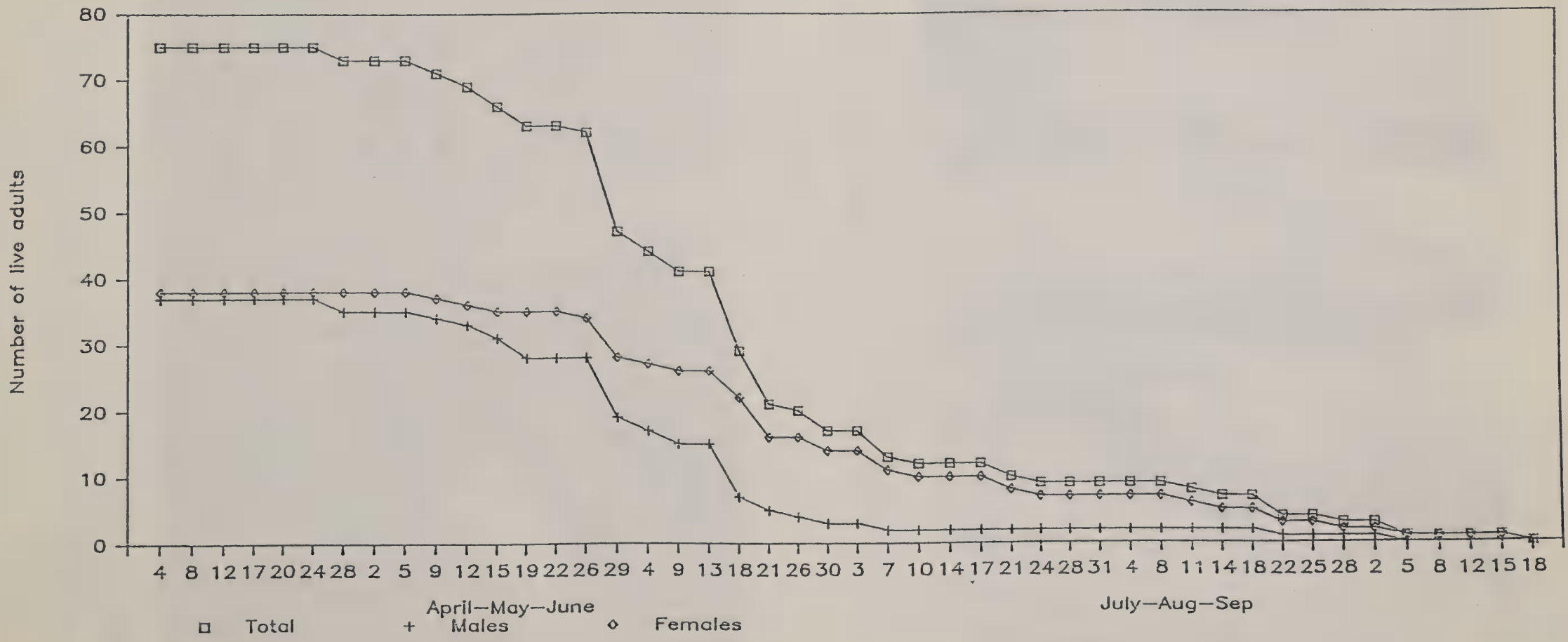



Fig. 8 : Number of live adults during the observations.

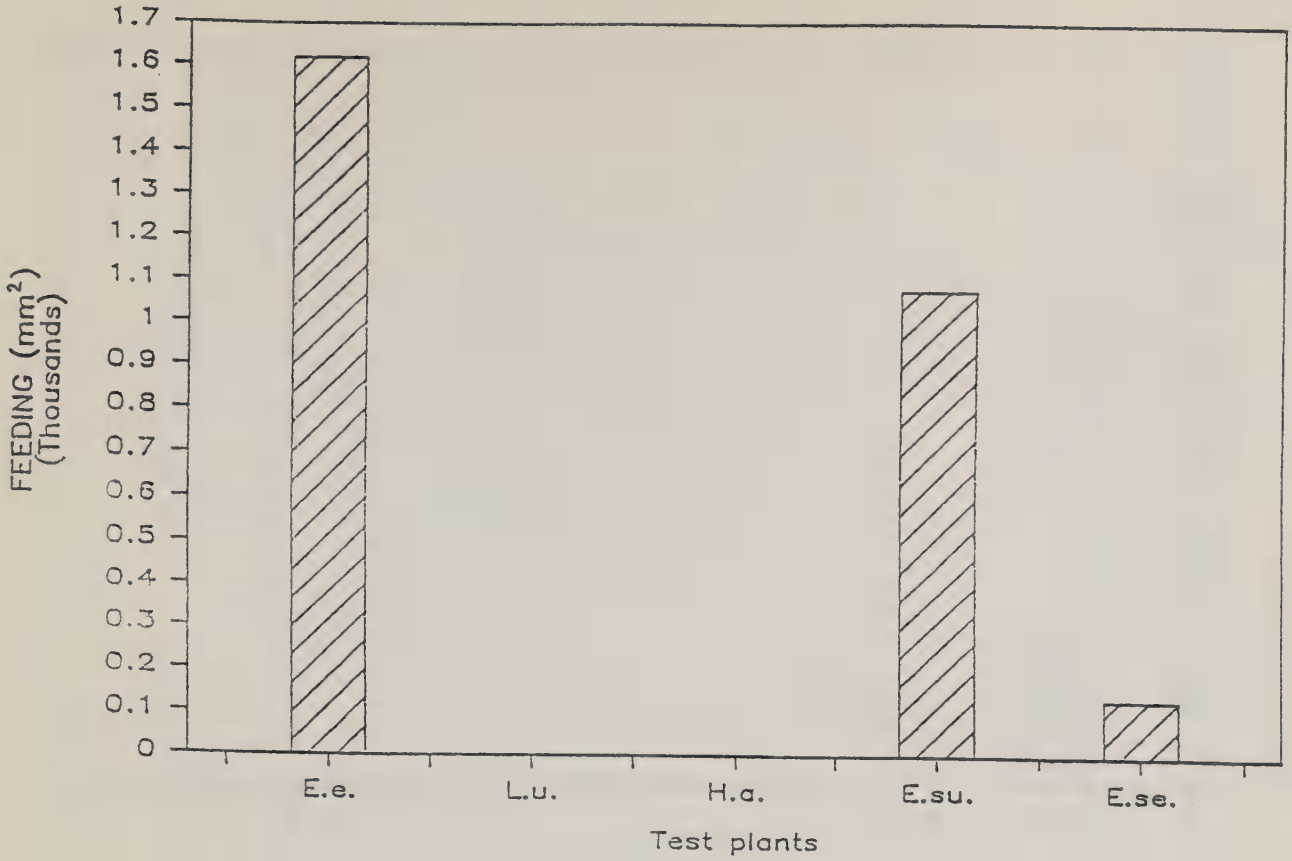



Fig. 9 : "No choice" feeding test: total consumption ($mm^2$) of leaves of plants tested.

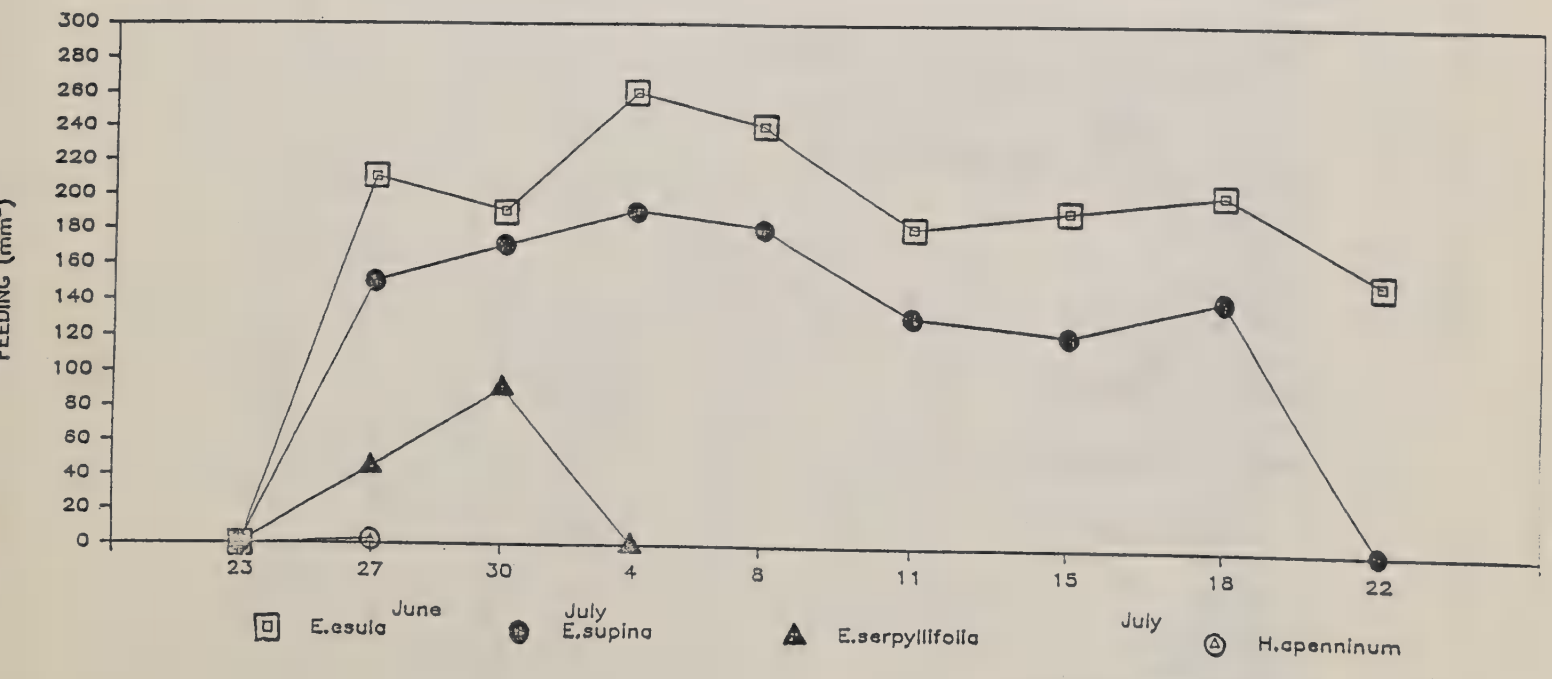



Fig. 10 : Feeding course in the No choice feeding test.

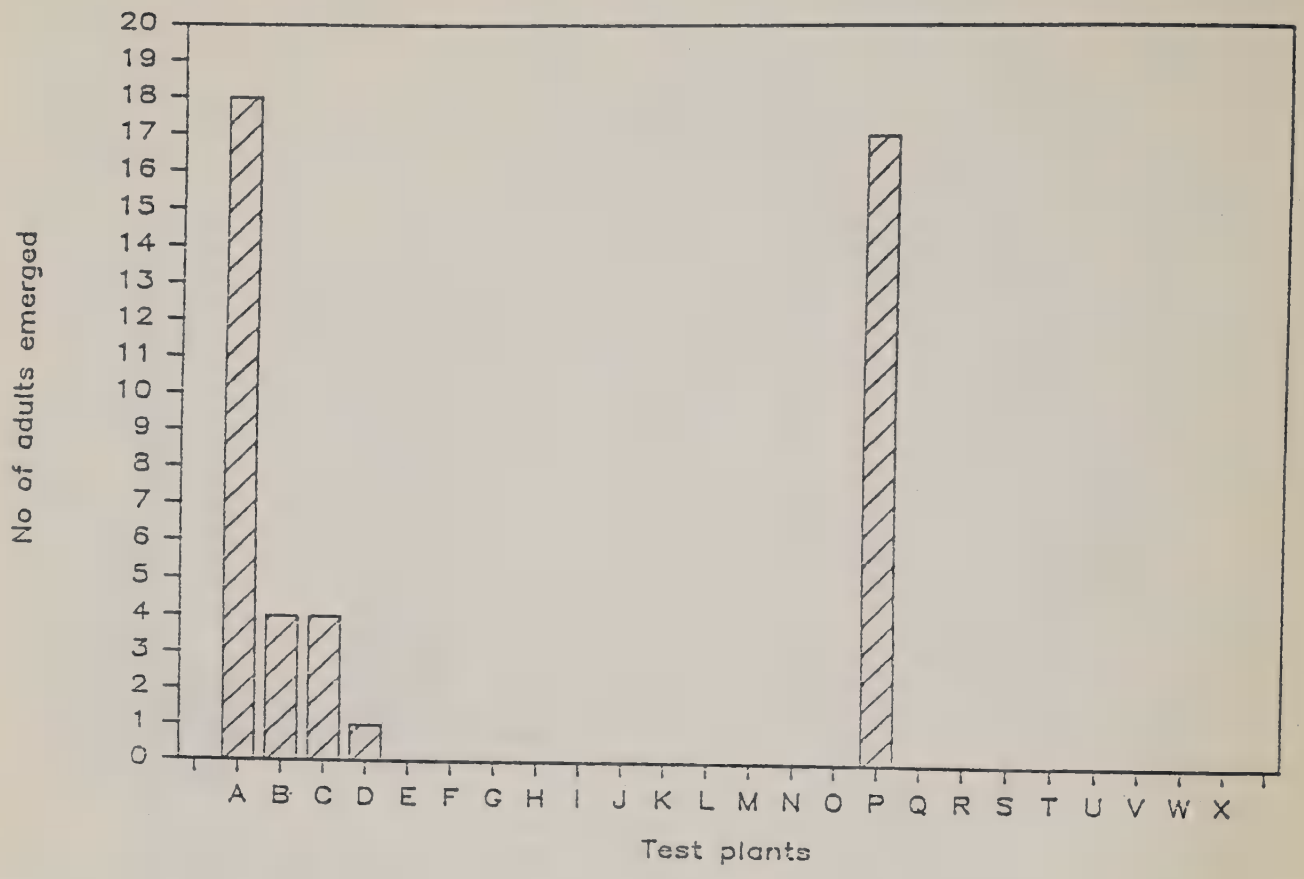



Fig. 11 : A. abdominalis adult emergence in the first instar larval survival test (25 larvae/species tested).

Legend (Fig.11)

A = Euphorbia esula from Pisa-Italy (Control)
B = E. esula-virgata from Montana-U.S.A.
C = E. esula-virgata from Nebraska-U.S.A.
D = E. esula-virgata from Wyoming-U.S.A.
E = Ricinus communis
F = E. trigona
G = E. milii
H = E. maculata
I = E. antisyphilitica
J = E. tirucalli
K = Codiaeum variegatum
L = E. characias
M = Linum usitatissimum
N = E. supina
O = E. serpyllifolia
P = E. lucida
Q = E. peplus
R = E. marginata
S = E. lathyris
T = Helianthemum apenninum
U = Pelargonium zonale
V = Lythrum salicaria
W = Ficus elastica
X = Ipomoea alba

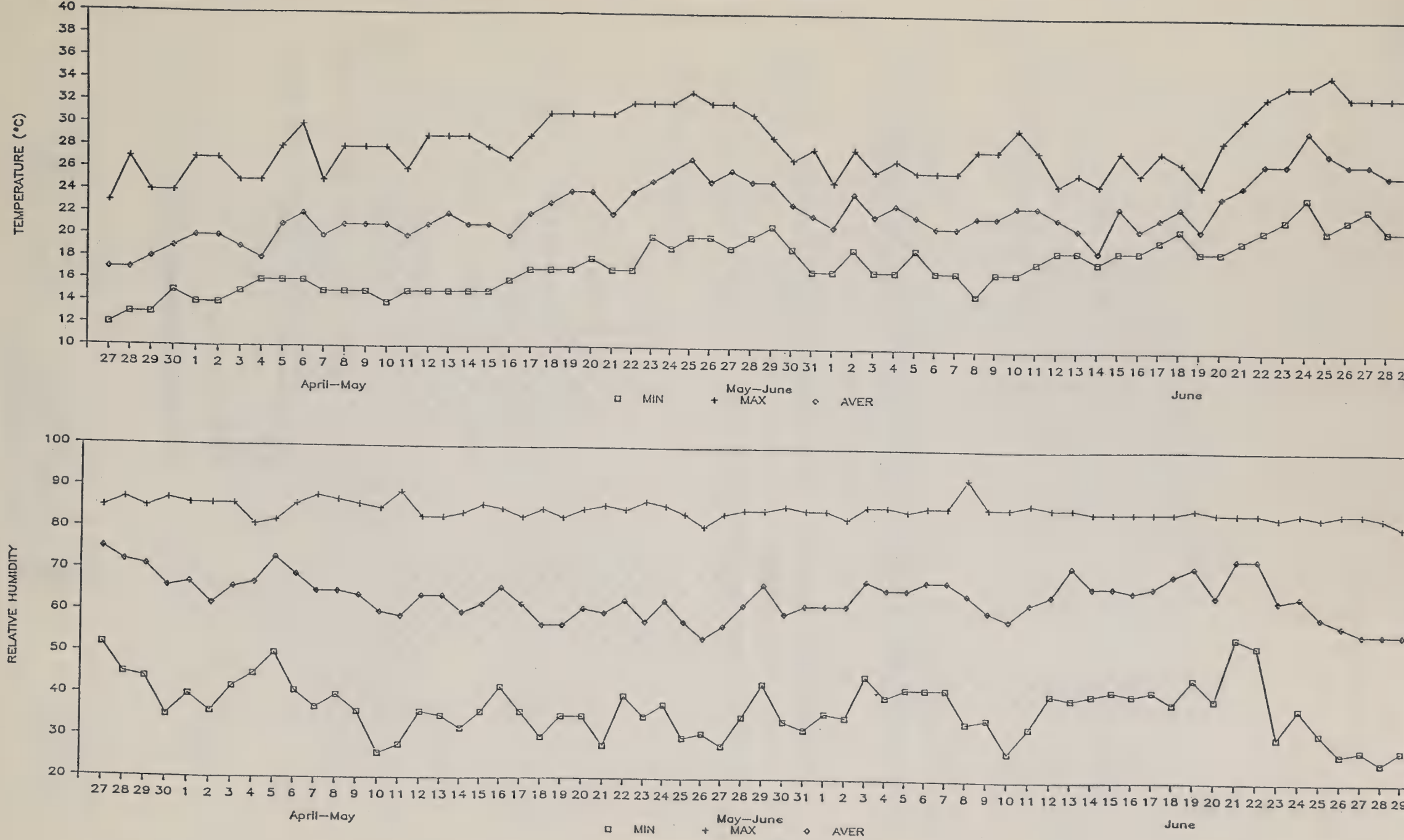



Fig. 12 : Temperature and humidity course during the Host suitability test.

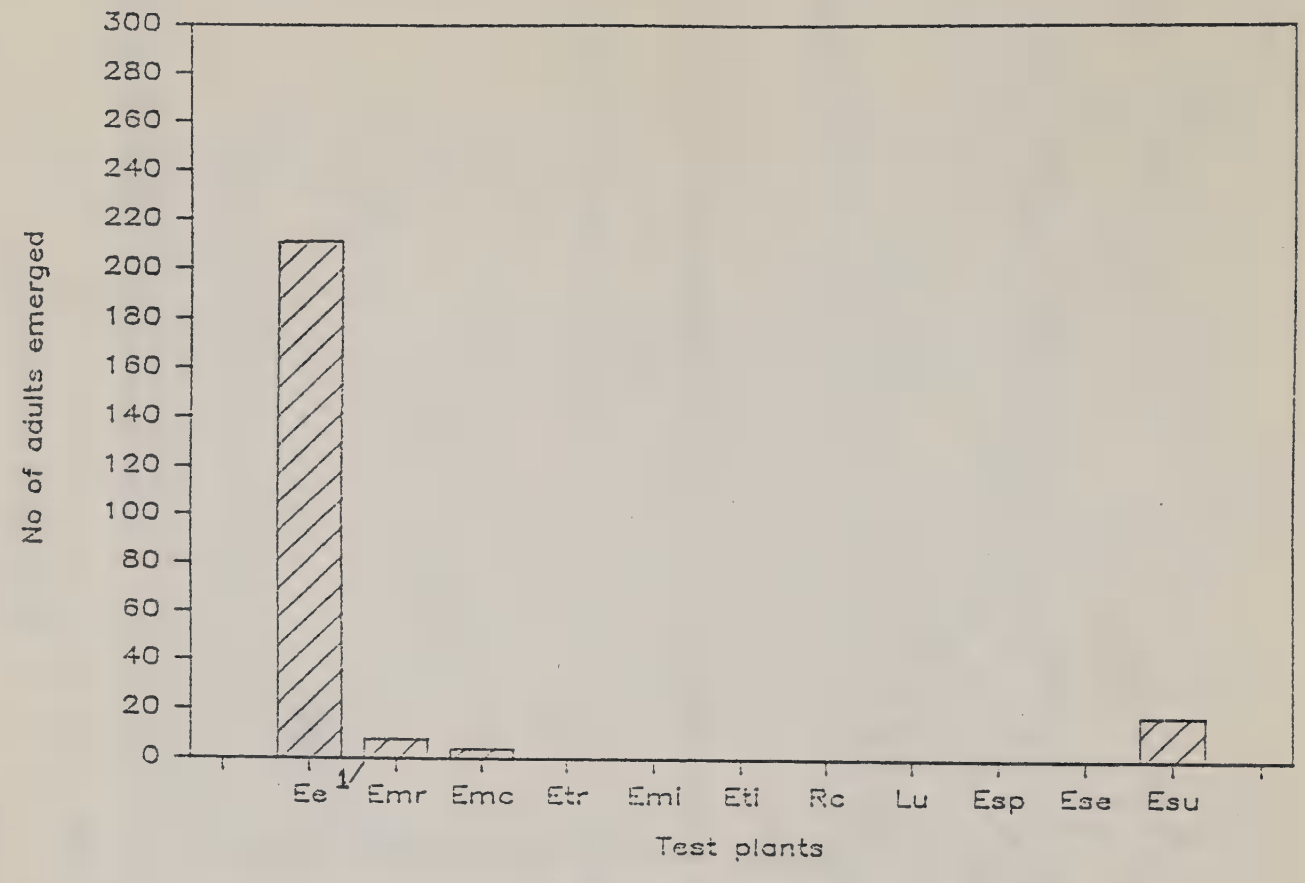



Fig. 13 : Host suitability trial: Multiple Choice (field, Plot 1).

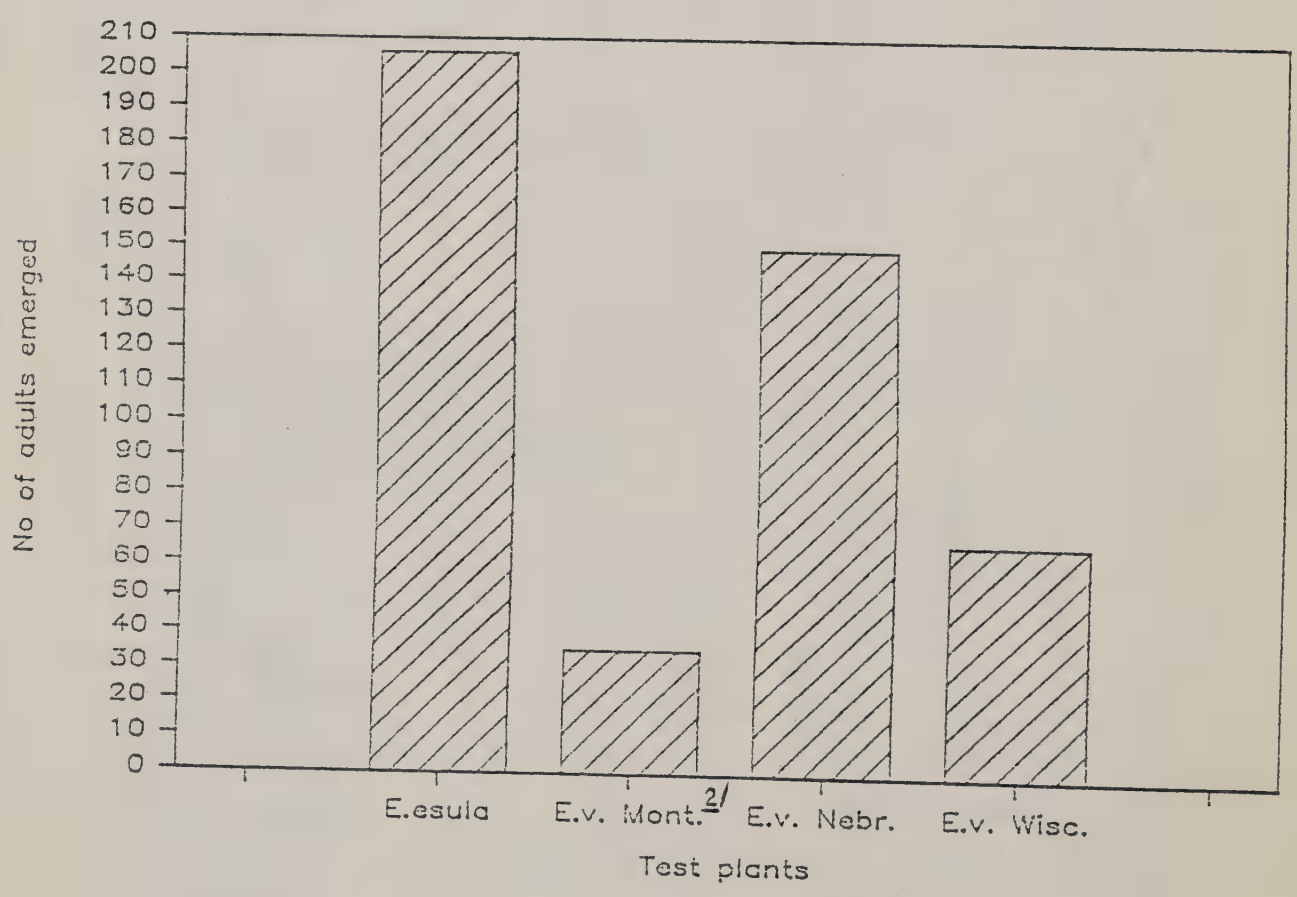



Fig. 14 : Host suitability trial: U.S. biotypes (field, Plot 2).

1/ Legend:

| | | |
|---|---|---|
| Ee = E. esula (Control) | Emi = E. milii | Esp = E. spatulata |
| Emr = E. marginata | Eti = E. tirucalli | Ese = E. serpyllifolia |
| Emc = E. maculata | Rc = Ricinus communis | Esu = E. supina |
| Etr = E. trigona | Lu = Linum usitatissimum | |

2/ E.v. = E. virgata

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## *Chamaesphecia* sp. (Lepidoptera: Sesidae)

(P. PECORA, M. STAZI, M. CRISTOFARO)

Specimens of *Chamaesphecia* sp. collected in Romania on *Euphorbia virgata* "group" were identified as *C. crassicornis* by K. Spatenka, Vùk PS, Peckg, Czechoslovakia. This species was described by Bartel in 1912 from specimens which came from Uralsk (Kazakhstan). It was also found in Eastern Austria (Sterzel 1967) and southern Czechoslovakia on *E. virgata* (Lastuvka, 1980). From infested roots of *E. virgata* "group", collected at the end of October 1985 near the Danube delta in Romania, only a few adults emerged in July 1986. These adults laid only unfertile eggs, so no host specificity tests were made. Another collection of infested roots, containing various stages of larvae of *C. crassicornis* was made in October 1986 in Rumania. If adults emerge and we get fertile eggs, a larval survival test will be conducted in 1987 on several closely related *Euphorbia* spp. and plants of economic importance.

### COLLECTIONS

*Bayeria capitigena* (Bremi)(Dipt.: Cecidomyiidae); (M. Stazi, M. Cristofaro collectors):

Five shipments of tip galls containing various instars of larvae and pupae of *B. capitigena* were shipped to Albany, California. These galls were collected on *E. esula* at S. Rossore, Italy, on: May 7 (170 galls), May 26 (200 galls), June 4 (310 galls), June 12 (230 galls) and July 3 (300 galls). Ten working days were required to make these collections.

Dasineura capsulae Kieffer (Dipt.: Cecidomyiidae); (M. Stazi, M. Cristofaro collectors):

About 10,000 mature larvae of D. capsulae were produced from 1300 galls collected on E. esula at S. Rossore in mid-June 1986. These larvae were transferred in proper containers to hibernate until next spring. Two working days were spent to collect this stock of galls.

Oberea erythrocephala Schrank (Col.: Cerambycidae); (M. Stazi, M. Cristofaro collectors):

Adults of this longhorned beetle, collected on E. esula at S. Rossore on May 27 (90 adults), June 4 (228 adults) and June 12 (200 adults), were shipped to Albany, California for field releases. Six working days were needed to collect these beetles.

Aphthona flava Guill. (Col.: Chrysomelidae); (M. Stazi, M. Cristofaro collectors):

Three shipments of this flea beetle were sent to Albany, California for field releases. These insects were collected on E. esula on June 5 (178 adults), June 12 (1100 adults) and July 3 (950 adults). Seven days were spent for these collections.

Aphthona cyparissiae (Koch) (Col.: Chrysomelidae); (P. Pecora, L. Fornasari collectors):

Three hundred fifty adults of A. cyparissiae were collected on E. cyparissiass near St. Polten (Austria) on June 20, 1986. On July 12 another 800 adults were collected in the same site. These insects were shipped to Albany CA. for field releases. Three days were necessary for these collections.

Aphthona czwalinae Weise (Col.: Chrysomelidae); (P. Pecora, L. Fornasari collectors):

Two shipments of this flea beetle, collected on E. esula in Eastern Austria on June 21 (350 adults) and July 13 (200 adults), were sent to Albany, California for further testing into quarantine. Four days were spent to make these collections.

# YELLOW STARTHISTLE PROJECT

## *Eustenopus hirtus* (Waltl, 1838) (Coleoptera: Curculionidae)

T. Mimmocchi and S.L. Clement

This year's research for the yellow starthistle (YST) project centered on *Eustenopus hirtus* (Waltl, 1838), a flowerhead weevil associated with YST in Greece and Turkey. Specific objectives were: (1) to collect more data on feeding and oviposition behavior on various test plant species under choice and no-choice laboratory conditions; (2) to investigate adult feeding oogenesis and oviposition behavior on cultivated safflower under no-choice laboratory conditions; (3) to study female reproductive behavior in the presence/absence of males; (4) to collect data on the reproductive output of single females; and (5) to gather information on larval development in the buds of safflower and other test plant species.

## METHODS AND RESULTS

### OBJECTIVE 1

*Study 1*: Adult weevils (n=182) were allowed to overwinter in plant debris in two outdoor cages. From this group only 41 survived the winter and these were used to collect more data on adult feeding, mating, and oviposition behavior. As beetles became active (around May 2) they were transferred from the overwintering cages to 100 cc. transparent plastic containers (12-13 beetles/ container) with fresh YST rosettes from the Rome Laboratory garden. Rosettes, held in water-filled vials, were changed as needed. Containers were held in a quarantine greenhouse (22.7 $\pm$6.5°C; 60-80% RH; natural lighting) to observe feeding mating, and oviposition.

A few beetles nibbled on rosette leaves. This feeding was first observed on May 6 and six days later, on May 12, we set up a no-choice feeding test with 34 beetles and 5 test plant species. Black organdy sleeve cages were placed over branches of test plant species with flower buds and 2 unsexed beetles were introduced into each cage. Observations were taken for 15 days. More details are given in Table 1.

Table 1 (Test A) shows that adults readily fed on the control plant (YST) and Centaurea nicaeensis. Adult survival was good except on Onopordum acanthium, where mortality was 83.3%. No eggs were laid on any of the plants.

Study 2: After the above 15-day observation period, surviving beetles were transferred to a 2000 cc. container and were given a mixture of YST bud stages (Bu 1 through 4). Buds were changed and dissected every 3-4 days to note the type of damage inflicted by feeding adults to each bud stage.

The following types of damage were observed: Bu-1's were almost completely consumed. In Bu-2 and 3's, feeding punctures were made and the achenes and receptacles were consumed. On B-4's, the preferred oviposition substrate of E. hirtus, light nibbling occurred. We observed the first pair of copulating beetles on May 28.

Study 3: A second no-choice feeding test (Test B) was conducted with additional test plants between June 4 and 11. Experimental conditions were similar to those of "Study 1" except that temperature was 20.2 $\pm$5.2°C. and the relative humidity was 35-85% rH. The weevils for this study came from the overwintering material.

Table 1 (Test B) shows that heavy feeding again occurred on YST. Beetles did not feed on buds of Scolymus hispanicus or Papver somniferum, but slight feeding occurred in safflower. Also a few of the very small buds of the Artemisia vulgaris were destroyed but the vast majority showed no signs of feeding damage.

Study 4: A large number of beetles were collected June 19 in northern Greece (Doirani) by R. Sobhian and P. H. Dunn, and arrived in Rome on June 27. The 407 survivors were allowed to feed for at least 48 hours on YST buds (obtained from Greek plants) before copulating pairs were selected for tests.

Weevils from the Doirani collection were used in a feeding-oviposition choice test made between June 29 and July 7. Branches of potted YST plants and test plants were tied together and caged within black organdy sleeve cages so beetles (2 mating pairs/cage) could have a choice of food and oviposition substrates. This test was conducted in the Rome quarantine greenhouse (temp. 23.7 ±4.9°C., 35-85% RH and natural lighting). Results are given in Table 2.

Heavy feeding and oviposition occurred only on YST and C. nicaeensis. Very little feeding took place on safflower and Cirsium arvensis while no feeding occurred on Cichorium intybus. Beetle survival was high and well-developed ovaries were noted in dead females (Table 2). It is important to stress that only minor feeding was recorded on safflower (only on leaves) when YST buds were present. (Fig. 1). Oviposition and egg hatching occurred only on YST (118 eggs, 17 larvae) and on C. nicaeensis (10 eggs). No eggs were found in the other test plant capitula (fig. 2).

OBJECTIVE 2

This study was done to measure beetle feeding oogenesis and oviposition on YST and safflower buds under confined conditions (i.e., on 500 cc. cup cage with organdy tops).

The study was started on June 30 in the quarantine greenhouse (temp. 23.9 ±4.9°C; 35-85% RH; natural night) with 40 mating pairs of beetles. Twenty couples (one/cup) were placed in 500 cc. cardboard cups with safflower buds (one bud 10-15 mm. dia./cup); the other 20 couples were placed in cups with YST buds (1 Bu-2, 1 Bu-3 and 1 Bu-4/cup). The cups were inspected daily to replace dead males. A replicate was terminated if a female died prematurely. (It was necessary to dissect dead beetles to determine their sex). Buds were changed every 3 days and the number of eggs laid was recorded.

On July 14 the study was terminated and all beetles were dissected to record the condition of ovaries and the number of developed eggs in females. The results of the feeding and oviposition trial are shown in Table 3 (Parts A & B) and the dissections of the female are found on table 4. The last two times that YST buds were changed we had to double the number of new buds offered because 3 buds proved to be insufficient for a 3-day period, i.e. the Bu-2 YST buds were almost completely destroyed and more mature buds suffered considerable damage. Some feeding damage was inflicted on safflower buds. Over a 15-day period, 18 females (two died prematurely and were not counted) laid 12.83 ±3.3 ($\bar{x}$ ±SD) eggs in YST buds (total of 231). No eggs were deposited in safflower buds.

All females in the YST treatment had well-developed ovaries at the end of the study (0.9 ±0.64 eggs/female; $\bar{x}$ ±SD). No eggs were found in females from the safflower treatment; their ovaries also showed signs of deterioration. Moreover, overall beetle mortality was 30% on safflower, but only 10% on YST.

OBJECTIVE 3

Another group of adults from the Doirani (Greece) collections was used to obtain more information on adult reproductive behavior. This study was conducted between June 30 and July 23 in the quarantine greenhouse (temp. 23.3 ±4.8°C; 35-85% RH; natural light).

Study 1: One study was designed to measure female oviposition in the presence and absence of males. To accomplish this, we placed males and females (2 couples/organdy sleeve cage) in a series of cages placed over branches with YST or safflower buds and only mated females (2 $^{\circ}$/cage) in another similar series of cages. There were 5 replicates and potted plants were used. Eggs and larvae were counted and midway through the study, when the cages were relocated over different buds, and once again at the end of the study.

Table 4 shows that eggs were only laid in YST buds. Over the course of 16 days the 10 females with males laid 14.60 ±2.38 ($\bar{x}$ ±SD) viable eggs while the 10 "single" females laid 16.00 ±2.82 viable eggs; these values are not significantly different (F=0.52; P=0.01). We tentatively concluded that multiple matings are not required for continued female oviposition.

Study 2: At the same time Study 1 was in progress, we also collected more feeding and oviposition data by caging 2 pairs of beetles on potted plants of C. nicaeensis, C. americana (North American native) and Scolymus hispanicus. Table 4 shows that buds of C. nicaeensis serve as an oviposition substrate for E. hirtus. The results also show that heavy feeding took place on C. americana, but that no eggs were laid on 5 plants of this species. Besides feeding on buds of C. americana, beetles fed on stems near the base of inflorescences and, in so doing, they affected bud and flowerhead development in this test plant. Mortality was 100% on Scolymus after about 5-6 days, but some minor leaf feeding was observed on this plant. Results of Eustenopus adult-feeding behavior, pooled from table 1 and 4, are illustrated in fig.3.

OBJECTIVE 4

A small scale study was conducted between July 1 and August 13 to collect more data on the rate of egg-laying by females over time. A mating couple was in a 500 cc. cup with a bouquet of 6 YST buds (2 Bu-2, 2 Bu-d and Bu-4), replicated 5 times. The buds were changed every 3 days to collect data on the number of eggs laid over a 3-day period. Cups were inspected each day and dead beetles were removed and dissected for sex determination. Only dead males were replaced. The study was conducted in the quarantine laboratory (temp. 23.7 ±4.6°C; 35-85% RH; natural light).

The weevils laid an average of 2.57 (±1.27) and 4.29 (±2.33) eggs/$^{\circ}$ in a 3-day period. A total of 163 eggs were laid. The results are presented in Table 5.

OBJECTIVE 5

Our technique of placing fertile eggs in safflower buds for 1st instar larvae survival trials was not very successful. The problem was the altered tissue in the hole made to insert the egg into the bud. When the egg hatched the larvae were confronted with dried or injured tissue not encountered in a normal oviposition hole. It may be necessary to try some new methods if this phase of the overall Eustenopus program is continued. However, one egg placed in a safflower bud did hatch and the larvae took 25 days to reach the pupal stage. Eight days later the pupa developed into an adult. This fragment of information confirms the work of R. Sobhian, who stated in earlier reports that Eustenopus larvae can complete their development if placed in safflower buds.

Eggs development data was also collected in the course of our work. This was done by transferring freshly laid eggs from YST buds to hatching containers (Rizza 1977) in a dark environmental chamber set at 25 ±1°C. Duration of the egg stage under these conditions was 5.6 ±0.75 ($\bar{x}$ ±SD) days n=25). A total of 489 eggs were transferred to hatching containers, and they were all viable. The oval, smooth eggs were 0.68 ±0.04 mm wide and 1.9 ±0.06 mm long ($\bar{x}$ ±SD) (n=60). They were pale yellow and translucent when laid but gradually turned darker yellow with age. A developing larva was visible under the chorion ca. 4 days after oviposition.

CONCLUDING REMARKS:

Two years of research results have now led us to conclude that E. hirtus is a potential biological control agent for YST in the western U.S. Therefore, more feeding and oviposition tests are planned for the 1987 season. In these tests, emphasis will be placed on testing about 20 plant species from the plant list approved by the Technical Advisory Group on Biological Weed Control.

## Literature Cited

Maddox, D., M., (1981). Introduction, phenology, and density of yellowstar thistle in coastal intercoastal and central valley situations in California. USDA, ARS, Agricultural Research Results, ARR-W-20, 33 pp.

Rizza, A., (1977). Comparison of *Phrydiuchus spilmani* and *P. tau*. Ann. Entom. Soc. Am. 70: 7-10.

Table 1: Summary of the results of a no-choice feeding test with overwintering adults of Eustenopus hirtus, sex unknown (two weevils per cage), Rome, Italy, May 12-June 11, 1986.

| Test | Test Plants (no.of plants) and Source of Seeds | Plant Stage | Min-Max No.of Buds Offered | Feeding Damage Rating[4] | Total No.Adults Beginning | Total No.Adults End |
|---|---|---|---|---|---|---|
| A[1] | Centaurea solstitialis (5) Lab.Garden, Rome, Italy | Bu-1[3] | 4-6 | 3 | 10 | 10 |
| | Centaurea cineraria (3) Bot. Garden, Trieste, Italy | Buds (8-13 mm)[5] | 3-5 | 0/1 | 6 | 5 |
| | Galactites tomentosa (3) Lab.Garden, Rome, Italy | Buds (10-15 mm) | 3-5 | 1 | 6 | 5 |
| | Onopordum acanthium (3) Castel del Monte (BA), Italy | Buds (20-30 mm) | 3-5 | 0 | 6 | 1 |
| | Centaurea nicaeensis (3) Lab. Garden, Rome, Italy | Buds (9-13 mm) | 10-16 | 2 | 6 | 6 |
| B[2] | Centaurea solstitialis (4) Lab. Garden, Rome, Italy | Bu-1, 2 | 6-10 | 3 | 10 | 9 |
| | Carthamus tinctorius (3) var. Hartman, U.S.A. | Small Buds (10-15 mm) | 4 | 1 | 6 | 5 |
| | Artemisia vulgaris (3) Bot. Garden, Finland | Small Buds (2-3 mm) | ? ? ?[6] | 0/3[7] | 6 | 5 |
| | Scolymus hispanicus (3) Lab. Garden, Rome, Italy | Small Buds (10-15 mm) | 5-6 | 0 | 6 | 3 |
| | Papaver somniferum (3) Bot. Garden, Padova, Italy | Buds (8-12 mm) | 1-2 | 0 | 6 | 5 |

1/ Test started on May 12, 1986 and was terminated 15 days later.
2/ Test started on June 4, 1986 and was terminated 7 days later.
3/ Classification according to Maddox (1981).
4/ Based on a scale of 0 to 3: 0= no feeding; 1= very little feeding, no effect on bud development; 2= very little feeding on well developed capitula, but considerable damage to young buds; 3= heavy damage, complete bud destruction.
5/ Diameter of buds.
6/ ? ? ? = too numerous to count.
7/ Majority of buds were not damaged, but a few were destroyed (hence rating of 0/3).

Table 2: Summary of the results of an adult feeding and oviposition choice test with Eustenopus hirtus, Rome, Italy, June 29- July 7, 1986.

| Rep. No. | Plants Offered to Beetles and Source of Seeds | YST No. of Buds [2/] | No. of Buds Test Plant | No. of Feeding Scars on YST | No.of Feeding Scars on Test Plant | No.of Eggs(E) and Larvae(L) Found on YST | No.of Eggs(E) and Larvae(L) Found on Test Plant | No. Dead Adults and Condition of Ovarioles at Dissection ♂ - ♀ |
|---|---|---|---|---|---|---|---|---|
| 1 | Centaurea solstitialis | 10 | 3[3/] | Buds Destroyed | 2 on leaves | 6E + 1L | 0 | 0 - 1 dev., no eggs [4/] |
| 2 | Thermi, Greece | 14 | 3 | Buds Destroyed | 5 on leaves | 8E | 0 | 0 - 0 |
| 3 | and | 12 | 3 | Buds Destroyed | 3 on leaves | 4E + 3L | 0 | 0 - 1 dev., 2 eggs |
| 4 | Carthamus tinctorius | 14 | 4 | Buds Destroyed | 9 on leaves | 9E | 0 | 1 - 0 |
| 5 | var. Hartman, U.S.A. | 11 | 3 | Buds Destroyed | 10 on leaves | 8E + 1L | 0 | 2 - 0 |
| 1 | Centaurea solstitialis | 15 | 21 | Buds Destroyed | 1 | 6E + 2L | 0 | 0 - 1 dev., no eggs |
| 2 | Thermi, Greece | 9 | 10 | Buds Destroyed | 0 | 4E | 0 | 0 - 0 |
| 3 | and | 12 | 18 | Buds Destroyed | 0 | 7E + 1L | 0 | 0 - 0 |
| 4 | Cirsium arvensis | 12 | 13 | Buds Destroyed | 0 | 8E + 1L | 0 | 0 - 0 |
| 5 | Lab.Garden, Rome, Italy | 14 | 14 | Buds Destroyed | 2 | 6E | 0 | 0 - 0 |
| 1 | Centaurea solstitialis | 10 | 22 | Buds Destroyed | 0 | 0 | 0 | 2 - 0 |
| 2 | Thermi, Greece | 15 | 35 | Buds Destroyed | 0 | 5E + 2L | 0 | 0 - 1 dev., no eggs |
| 3 | and | 10 | 31 | Buds Destroyed | 0 | 4E + 1L | 0 | 0 - 0 |
| 4 | Cichorium intybus | 18 | 35 | Buds Destroyed | 0 | 6E | 0 | 0 - 1 dev., no eggs |
| 5 | Lab.Garden, Rome, Italy | 18 | 40 | Buds Destroyed | 0 | 8E | 0 | 0 - 0 |
| 1 | Centaurea solstitialis | 12 | 11 | Buds Destroyed | 4[5/] | 4E + 2L | 0 | 1 - 1 dev., no eggs |
| 2 | Thermi, Greece | 13 | 12 | Buds Destroyed | 2 | 6E | 3E | 0 - 0 |
| 3 | and | 12 | 10 | Buds Destroyed | 4 | 5E + 2L | 4E | 0 - 1 dev., no eggs |
| 4 | Centaurea nicaeensis | 18 | 10 | Buds Destroyed | 5 | 6E | 3E | 0 - 0 |
| 5 | Lab.Garden, Rome, Italy | 16 | 15 | Buds Destroyed | 6 | 8E + 1L | 0 | 1 - 0 |

1/ Two pairs (2♀;2 ♂) of beetles were coupled .
2/ Mixture of bud stages Bu-1,2 and 3 was offered. (Classification according to Maddox, 1981).
3/ Buds 15-20 mm in diameter.
4/ Dev., no eggs: means ovarioles developed but no eggs found.
5/ The small buds of C. nicaeensis (similar to YST Bu-2) were completely destroyed, while light damage was inflicted to larger buds.

Table 3: Summary of the results of a no-choice adult feeding and oviposition test with Eustenopus hirtus, laboratory study, Rome, Italy, June 30 - July 14, 1986.

Part A: Feeding and oviposition substrate: Centaurea solstitialis buds (YST) in a 500 cc cardboard cup with one couple (1 ♀, 1 ♂).

| Rep. No. | Change #1 [1] | | | | Change #2 | | | | Change #3 | | | | Change #4 | | | | Change #5 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | No. Eggs Laid | Feeding Damage [2] Bu-2 | Bu-3 | Bu-4 | No. Eggs Laid | Feeding Damage Bu-2 | Bu-3 | Bu-4 | No. Eggs Laid | Feeding Damage Bu-2 | Bu-3 | Bu-4 | No. Eggs Laid | Feeding Damage Bu-2 | Bu-3 | Bu-4 | No. Eggs Laid | Feeding Damage Bu-2 | Bu-3 | Bu-4 |
| 1. | 2 | d [3] | 2 | 0 | 2 | d | 2 | 4 | 0 | d | d | 2 | 1 | d | 4 | 2 | 4 | 5 | 3 | 4 |
| 2. | 3 | d | 2 | 1 | 2 | d | d | 2 | 3 | d | d | 4 | 2 | d | 3 | 4 | 4 | d | 4 | 4 |
| 3. | 0 | d | 0 | 5 | 3 | d | d | 4 | 3 | d | d | 6 | 3 | d | 3 | 2 | 3 | 4 | 3 | 2 |
| 4. | 1 | d | 1 | 3 | 4 | d | d | 4 | 3 | d | d | 3 | 5 | d | 2 | 4 | 6 | 2 | 4 | 3 |
| 5. | 2 | d | 0 | 2 | 1 | d | 3 | 3 | 3 [7] | d | d | 5 | 2 | d | 3 | 2 | 5 | d | 6 | 4 |
| 6. | 0 | d | 5 | 1 | 2 | d | 3 | 3 | 2 | d | d | 6 | 1 | d | 3 | 1 | 2 | d | 3 | 5 |
| 7. | 0 | 0 | 0 | 2 | 0 | 1 | 2 | 2 | 1 [7] | d | 0 | 2 [4] | | | | | | | | |
| 8. | 1 | d | 2 | 0 | 2 | d | 3 | 5 | 2 | d | 3 | 3 | 2 | d | 6 | 5 | 4 | 1 | 4 | 6 |
| 9. | 3 | d | 2 | 2 | 1 | d | 2 | 4 | 2 [7] | d | 4 | 2 | 3 | d | 5 | 5 | 0 | 2 | 5 | 5 |
| 10. | 1 | d | 4 | 4 | 3 | d | 3 | 4 | 4 | d | 4 | 5 | 2 | d | 7 | 6 | 4 | d | 7 | 6 |
| 11. | 1 | d | 4 | 3 | 2 | 2 | 3 | 2 | 3 | d | 3 | 5 | 4 | d | 4 | 5 | 1 | 4 | 2 | 3 |
| 12. | 2 | d | 0 | 3 | 2 | d | 3 | 4 | 3 | d | d | 4 | 5 | d | 3 | 4 | 5 | 2 | 4 | 4 |
| 13. | 0 [6] | d | 2 | 3 | 2 | d | 3 | 3 | 3 | d | d | 5 | 2 | d | 2 | 2 | 2 [8] | 1 | 3 | 2 |
| 14. | 1 | d | d | 1 | 3 | d | 3 | 4 | 4 | d | d | 5 | 4 | d | 4 | 2 | 2 | d | 1 | 1 |
| 15. | 2 | d | 2 | 2 | 2 | d | 4 | 3 | 2 | d | d | 5 | 4 | d | 3 | 3 | 4 | 2 | 3 | 2 |
| 16. | 2 | 2 | 4 | 4 | 2 | d | 4 | 2 | 1 | d | d | 3 | 5 | 2 | 6 | 5 | 4 | d | 4 | 3 |
| 17. | 3 | 1 | 1 | 4 | 2 | 2 | 2 | 3 | 3 | d | 4 | 3 | 3 | d | 4 | 2 | 4 | d | 3 | 2 |
| 18. | 0 | d | 2 | 0 | 1 | 1 | 2 | 2 | 3 | d | 3 | 5 | 3 | d | 3 | 2 [5] | | | | |
| 19. | 2 | d | 2 | 1 | 1 | d | 2 | 4 | 2 | d | 3 | 6 | 3 | d | 3 | 2 | 2 | 4 | 2 | 3 |
| 20. | 1 | d | 3 | 2 | 2 | d | 3 | 3 | 3 | d | d | 4 | 6 | d | 7 | 6 | 4 | d | 3 | 4 |

Table 3: continued.

Part B: Feeding and oviposition substrate: Carthamus tinctorius var. Hartman, U.S.A. buds[9] (SF) in a 500 cc cardboard cup with one couple (1 ♀,1 ♂).

| Rep. No. | Change #1 | | Change #2 | | Change #3 | | Change #4 | | Change #5 | |
|---|---|---|---|---|---|---|---|---|---|---|
| | No.Eggs Laid | No. of Feeding Punctures | No.Eggs Laid | No. of Feeding Punctures | No.Eggs Laid | No. of Feeding Punctures | No.Eggs Laid | No. of Feeding Punctures | No.Eggs Laid | No. of Feeding Punctures |
| 1. | 0 | 1 on leaf | ♂[10] | 1 on flowerhead | 0 | 3 on flowerhead | ♂[13] | 1 on leaf | ♂[15] | 1 on flowerhead |
| 2. | 0 | 0 | 0 | 3 on flowerhead | 0 | 0 | 0 | 5 on flowerhead | 0 | 1 on flowerhead |
| 3. | 0 | 1 on flowerhead | ♂[10] | 1 on flowerhead | | | | | | |
| 4. | 0 | 0 | 0 | 8 on flowerhead | 0 | 1 head/3 leaves | ♂[13] | 3 on flowerhead | 0 | 2 on flowerhead |
| 5. | 0 | 2 on leaf | ♂[10] | 0 | 0 | 0 | 0 | 2 head/3 leaf | ♂[15] | 2 on flowerhead |
| 6. | 0 | 2 on leaf | 0 | 3 on leaf | 0 | 0 | ♂[13] | 0 | | |
| 7. | 0 | 0 | 0 | 1 on flowerhead | 0 | 2 on flowerhead | 0 | 0 | 0 | 2 on flowerhead |
| 8. | 0 | 0 | 0 | 5 on flowerhead | 0 | 2 on flowerhead | 0 | 1 head/ 2 leaves | 0 | 4 on flowerhead |
| 9. | 0 | 3 on flowerhead | 0 | 4 on flowerhead | 0 | 2 on flowerhead | 0 | 1 on flowerhead | 0 | 0 |
| 10. | 0 | 2 on flowerhead | 0 | 1 on flowerhead | 0 | 0 | ♂[13] | 0 | | |
| 11. | 0 | 0 | 0 | 0 | 0 | 1 on flowerhead | 0 | 1 on flowerhead | 0 | 0 |
| 12. | 0 | 0 | 0 | 4 on flowerhead | ♂[12] | 5 on flowerhead | 0 | 2 on flowerhead | 0 | 1 on flowerhead |
| 13. | 0 | 0 | 0 | 2 on flowerhead | | | | | | |
| 14. | 0 | 2 on leaf | 0 | 2 on leaf | 0 | 2 on flowerhead | 0 | 2 on flowerhead | 0 | 0 |
| 15. | 0 | 1 on flowerhead | 0 | 2 on flowerhead | 0 | 2 on flowerhead | 0 | 2 on flowerhead | 0 | 0 |
| 16. | 0 | 0 | 0 | 2 on flowerhead | 0 | 3 on flowerhead | ♂[13] | 0 | | |
| 17. | 0 | 1 on leaf | 0 | 2 on flowerhead | 0 | 1 on flowerhead | 0 | 3 on flowerhead | 0 | 1 on flowerhead |
| 18. | 0 | 2 on leaf | 0 | 2 on flowerhead | ♂[12] | 0 | 0 | 2 on flowerhead | 0 | 0 |
| 19. | 0 | 0 | ♂[11] | 5 on leaf | | | | | | |
| 20. | 0 | 2 on leaf | 0 | 4 on flowerhead | 0 | 1 on flowerhead | ♂[14] | 4 on flowerhead | 0 | 0 |

1/ Change #1, #2, #3 : YST bouquets made of 1 Bu-2, 1 Bu-3, 1 Bu-4, changed every three days;
Change #4, #5 : YST bouquets made of 2 Bu-2, 2 Bu-3, 2 Bu-4, changed every three days;
2/ Feeding damage expressed as number of feeding punctures on buds offered.
3/ d = Buds completely destroyed.
4/ YST #7 : female died on 8/7.
5/ YST #18 : female died on 10/7.
6/ YST #13 : male recovered dead on 2/7 and replaced on the same date.
7/ YST #5, #7, #9 : males recovered dead on 7/7 and replaced on the same date.
8/ YST #13 : male recovered dead on 12/7 and replaced on the same date.
9/ One Safflower bud (diam. 10-15 mm) per bouquet changed every three days.
10/ SF #1, SF#5 : males recovered dead on 4/7 and replaced on the same date; SF #3: female died on 4/7.
11/ SF #13: female died on 5/7; SF #19: couple died on 5/7.
12/ SF #12, #18 : males recovered dead on 7/7 and replaced on the same date.
13/ SF #1, SF #4, SF #6: males recovered dead on 10/7 and replaced on the same date; SF #10, SF #16: female died on 10/7.
14/ SF #19 : male recovered dead on 11/7 and replaced on the same date.
15/ SF #1: couple died on 13/7; SF #5: male recovered dead on 13/7 and replaced on the same date.

Table 4: Summary of the results of a study on the reproductive behaviour, oogenesis, egg output and feeding of females (with or without males) of Eustenopus hirtus . Rome, Italy, June 30 - July 23, 1986.

| Rep. No. | Test Plant and Source of Seeds | No. of Buds Offered at Bud Changes 1/ | | No. of Eggs(E) and Larvae(L) found in Bud Dissections at the two Changes: | | No. of Eggs in Ovarioles at Dissection | | Condition of Ovarioles at Final Dissection | Duration of Test (in Days) | Feeding Damage Rating 2/ |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 1 | 2 | ♀ #1 | ♀ #2 | | | |
| | | | | | STUDY 1 | | | | | |
| 1 | Centaurea solstitialis | 12 | 19 | 6E | 12E+2L | 1 | 1 | developed | 16 | 3 |
| 2 | Thermi, Greece | 12 | 18 | 7E | 6E+1L | 1 | 1 | developed | 16 | 3 |
| 3 | | 14 | 10 | 5E | 4E+3L | 1 | 1 | developed | 16 | 3 |
| 4 | (♀♀ + ♂♂) | 11 | 13 | 4E | 9E+2L | 1 | 0 | developed | 16 | 3 |
| 5 | | 10 | 10 | 5E | 4E+3L | 1 | 0 | developed | 16 | 3 |
| 1 | Centaurea solstitialis | 10 | 27 | 7E | 6E+4L | 1 | 1 | developed | 16 | 3 |
| 2 | Thermi, Greece | 12 | 20 | 4E | 12E+1L | 1 | 1 | developed | 16 | 3 |
| 3 | | 21 | 12 | 5E | 12E+1L | 1 | 1 | developed | 16 | 3 |
| 4 | (only ♀♀) | 12 | 13 | 6E | 8E+3L | 1 | 1 | developed | 16 | 3 |
| 5 | | 11 | 11 | 5E | 6E | 1 | 1 | developed | 16 | 3 |
| 1 | Carthamus tinctorius | 4 | | 0 | 3/ | 1 | 0 | developed | 10 | 1 |
| 2 | var. Hartman, U.S. | 5 | | 0 | | 0 | 0 | not developed | 10 | 1 |
| 3 | | 5 | | 0 | | 0 | 0 | not developed | 10 | 1 |
| 4 | (♀♀ + ♂♂) | 5 | | 0 | | 0 | 0 | not developed | 10 | 1 |
| 5 | | 4 | | 0 | | 0 | 0 | not developed | 10 | 1 |
| 1 | Carthamus tinctorius | 4 | | 0 | 3/ | 0 | 0 | not developed | 11 | 1 |
| 2 | var. Hartman, U.S. | 5 | | 0 | | 0 | 0 | not developed | 12 | 1 |
| 3 | | 5 | | 0 | | 0 | 0 | not developed | 10 | 1 |
| 4 | (only ♀♀) | 7 | | 0 | | 0 | 0 | not developed | 11 | 1 |
| 5 | | 4 | | 0 | | 0 | 0 | not developed | 12 | 1 |
| | | | | | STUDY 2 | | | | | |
| 1 | Centaurea nicaeensis 5/ | 11 | 10 | 0 | 0 | 0 | 0 | developed | 23 | 2 |
| 2 | Minervino (Puglia), Italy | 17 | 11 | 1E | 2E+1L | 0 | 1 | developed | 23 | 2 |
| 3 | | 10 | 16 | 0 | 1E | 0 | 1 | developed | 23 | 2 |
| 4 | (♀♀ + ♂♂) | 12 | 13 | 0 | 1E | 0 | 0 | developed | 23 | 2 |
| 5 | | 6 | 11 | 0 | 0 | 0 | 0 | developed | 23 | 2 |
| 1 | Centaurea americana 5/ | 5 | 3 | 0 | 0 | 0 | 0 | developed | 22 | 3 |
| 2 | Texas, U.S.A. | 4 | 4 | 0 | 0 | 0 | 0 | developed | 18 | 3 |
| 3 | | 4 | 6 | 0 | 0 | 0 | 0 | developed | 22 | 3 |
| 4 | (♀♀ + ♂♂) | 5 | 0 | 0 | 3/ | 0 | 0 | developed | 9 | 3 |
| 5 | | 5 | 9 | 0 | 0 | 0 | 0 | developed | 21 | 3 |
| 1 | Scolymus hispanicus 5/ | 17 | | 0 | 4/ | 0 | 0 | not developed | 5 | 1 |
| 2 | Lab. Garden, Rome, Italy | 11 | | 0 | | 0 | 1 | developed | 5 | 1 |
| 3 | | 10 | | 0 | | 0 | 0 | not developed | 5 | 1 |
| 4 | (♀♀ + ♂♂) | 11 | | 0 | | 1 | 0 | developed | 6 | 1 |
| 5 | | 7 | | 0 | | 0 | 0 | not developed | 5 | 1 |

1/ Buds were changed about half way through the study (8-10 days after start of test per plant species).
2/ Based on a scale of 0 to 3: 0= no feeding; 1= very little feeding, no effect on bud development; 2= very little feeding on well developed capitula, but considerable damage to young buds; 3= heavy damage, complete bud destruction.
3/ Plants not available.
4/ Test terminated because all of the beetles died.
5/ Test run with males and females.

Table 5: Reproductive output of single females (**) of Eustenopus hirtus, July 1-August 13, 1986.

| Female No. | Number of Eggs Laid over Time | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 7/3 | 7/6 | 7/9 | 7/12 | 7/15 | 7/18 | 7/21 | 7/24 | 7/27 | 7/30 | 8/3 | 8/7 | 8/10 | 8/13 |
| ♀ #1 | 4 | 3 | 4 | 3 | 1 | 2 | 1 1/ | | | | | | | |
| ♀ #2 | 3 | 4 | 4 | 6 | 3 | 2 | 2 | 4(*) | 7 | 9 | 6 | 6 | 4 | 0 2/ |
| ♀ #3 | 4 | 5 | 1 | 5 | 3 | 4 | 1 3/ | | | | | | | |
| ♀ #4 | 4 | 2 | 5 | 3 | 3 | 2 | 4 | 3 | 2 4/ | | | | | |
| ♀ #5 | 3 | 3 | 4 | 5 | 3 | 4(*) | 4 | 3 | 2 | 3 | 0 5/ | | | |

1/ ♀ #1 died on 7/20: no eggs in ovarioles.
2/ ♀ #2 died on 8/13: no eggs in ovarioles.
3/ ♀ #3 died on 7/20: 1 egg in ovarioles.
4/ ♀ #4 died on 7/26: 1 egg in ovarioles.
5/ ♀ #5 died on 8/ 3: 1 egg in ovarioles.
(*) Male found dead and replaced on the same date.
(**) The experiment was set up on July 1 with one mating couple per cup (500 cc). A bouquet of 6 YST buds (2 Bu-1, 2 Bu-2, 2 Bu-3) changed and dissected every three days, was provided as feeding and oviposition substrates.

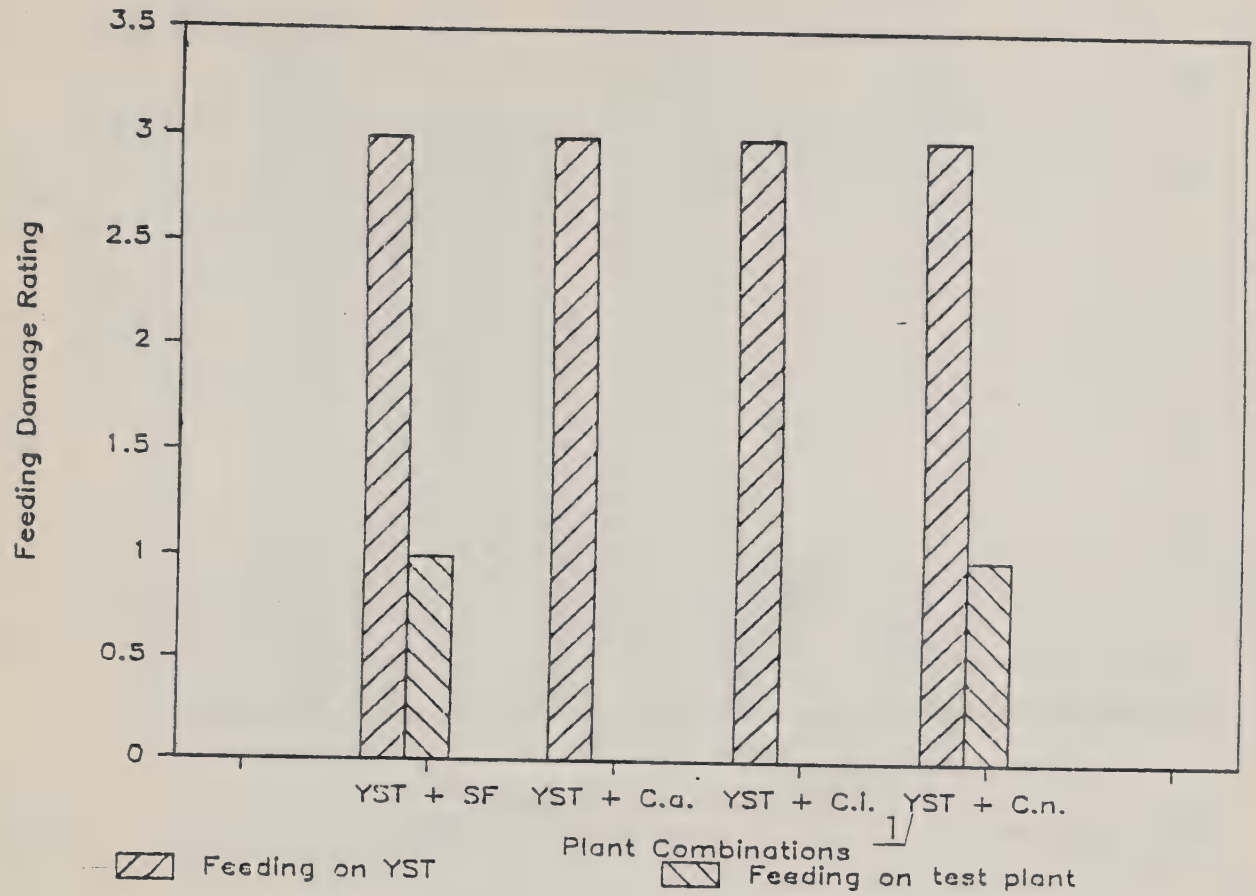


Fig. 1: Histogram showing the results of a choice feeding test, Rome laboratory, Italy, June 29 - July 7, 1986.

1/ YST + SF = YST + Carthamus tinctorius
YST + C.a.= YST + Cirsium arvense
YST + C.i.= YST + Cichorim intybus
YST + C.n.= YST + Centaurea nicaeensis

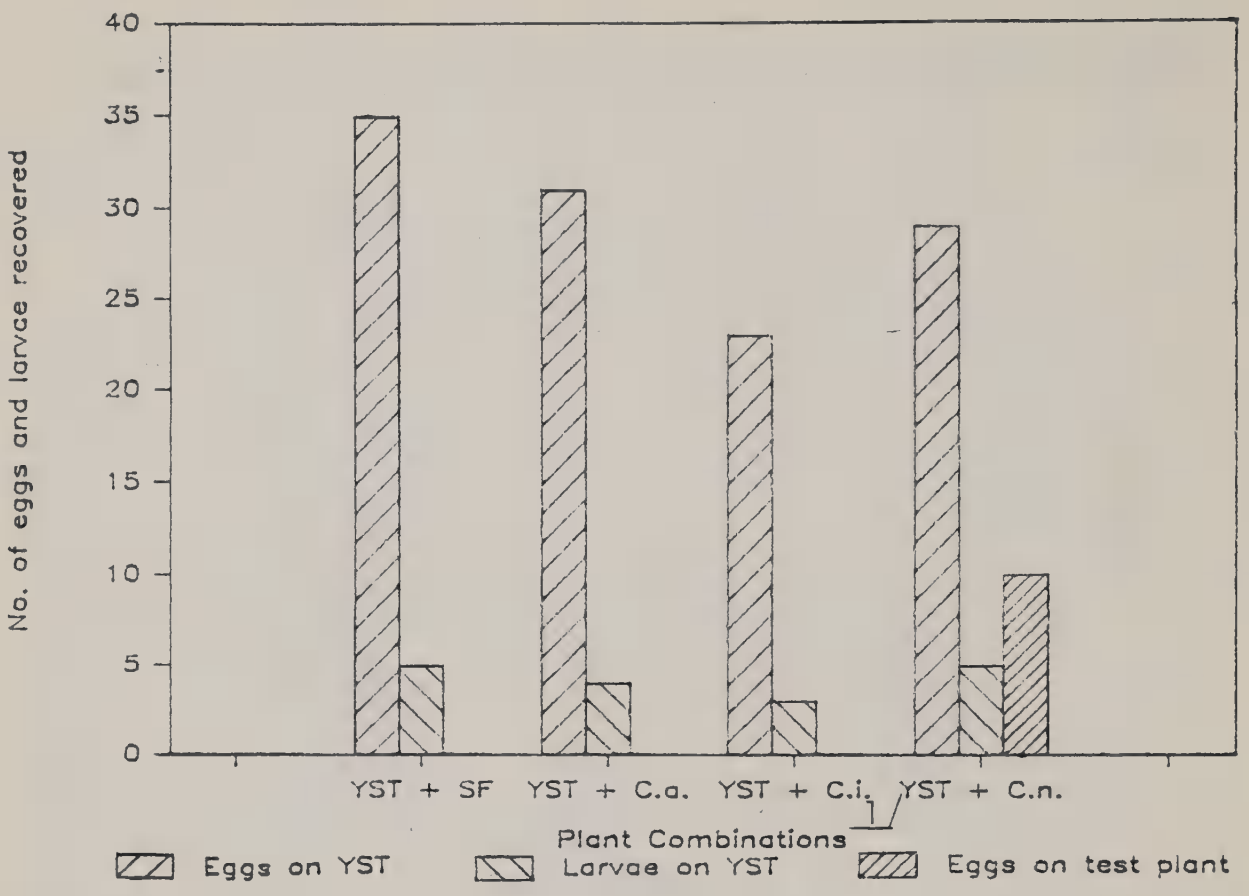


Fig. 2: Histogram showing the results of the oviposition choice test, Rome laboratory, Italy, June 29 - July 7. 1986.

1/ YST + SF = YST + Carthamus tinctorius
YST + C.a.= YST + Cirsium arvense
YST + C.i.= YST + Cichorium intybus
YST + C.n.= YST + Centaurea nicaeensis

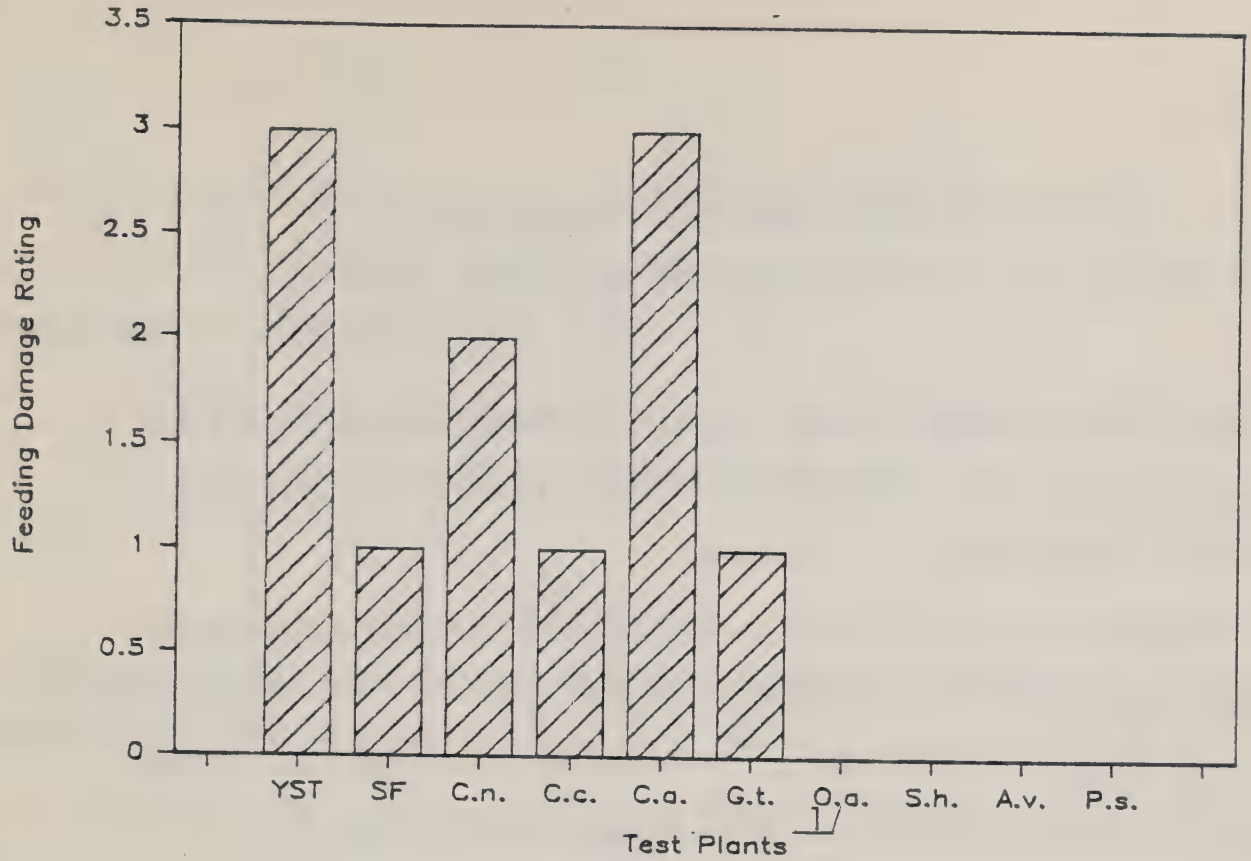


Fig. 3: Histogram showing the result of a no-choice feeding test with Eustenopus hirtus, Rome laboratory, Italy, May 12 - June 11,1986. Feeding Damage rating is based on a scale from 0 to 3: 0=no feeding; 1=very little damege, no effect on bud development; 2=very little feeding on well developed capitula, but considerable damage to young buds; 3=heavy damage, complete bud destruction.

1/

YST = Centaurea solstitialis
SF = Carthamus tinctorius
C.n.= Centaurea nicaeensis
C.c.= Centaurea cineraria
C.a.= Centaurea americana
G.t.= Galactites tomentosa
O.a.= Onopordum acanthium
S.h.= Scolymus hispanicus
A.v.= Artemisia vulgaris
P.s.= Papaver sonniferum

# KNAPWEED COLLECTION PROGRAM 1986

P. H. Dunn and G. Campobasso

Collecting trips were made in Northern Greece and Southern Italy to provide insects and plant material for (USDA), Albany (CIBC) Delémont, and (USDA) Rome laboratories.

GREECE: Between May 15 and June 2, collections of the root boring moth Pterolonche inspersa Stgr. (Lep: Pterolonchidae) and the seed-feeding weevil Bangasternus fausti Reitter (Coleop: Curculionidae) were made in Northern Greece revisiting known collecting sites around Thessaloniki.

## Pterolonche inspersa

Infested roots (n=120) of Centaurea diffusa containing P. inspersa were dug up at Thermi, Greece and brought to the USDA Laboratory there, where they were dissected under microscope. A total of 150 medium-size larvae were found and placed individually into 50 cc. plastic cups of artificial diet furnished by Dr. D. Schroeder of Delémont Laboratory (CIBC). Once the larvae accepted the artificial diet, the containers were packed and shipped to Albany, California. Seven days were spent by one person to make this collection.

## Bangasternus fausti

One thousand three-hundred adults of B. fausti were collected by two people at Panorama and Thermi between May 23 and June 1. Using USDA Laboratory facilities, the insects were separated according to sex and sent to the Rome Laboratory to start the host specificity and biology studies.

# A petition for the introduction into quarantine for testing Bangasternus fausti (Reitter) (Coleoptera: Curculionidae); a potential biocontrol agent of diffusa knapweed (Centaurea diffusa Lam.)

PAUL H. DUNN AND GAETANO CAMPOBASSO

## INTRODUCTION

Centaurea diffusa Lam., diffuse knapweed, is an introduced weed of European origin that is spreading throughout the uncultivated dry lands of Western Canada and the North-Western United States. It was first discovered in the United States in 1907 in an alfalfa field at Bingen, Klickitat County, Washington by Suksdorf (Watson an Renney 1974, citing Howell 1959). It has spread very rapidly and by 1979 was reported to cover 756,000 acres in Washington, 750,000 acres in Idaho (Maddox 1979).

The main economic loss from diffuse knapweed is from the reduction in high quality forage wherever it infests rangeland. The weed itself has little nutritive value and a high fiber content. Its dense, spiny overstory may reduce the availability of more desirable species. Also, it has an allelopathic effect on other plants. Watson and Renney (1974) reported that infested land produced 112 kg/ha of forage compared to the 896.8 kg/ha for noninfested land. The annual cost of the loss in carrying capacity of the 750,000 infested Oregon acres was estimated to be $600,000 (Maddox 1979). Maddox (1979) reports that the high levels of diffuse knapweed consumption can cause toxic symptoms in grazing livestock, especially horses.

The beneficial attributes of diffuse knapweed given by Watson and Renney (1974) are its ability, as a pioneer species, to rapidly establish plant cover on barren soil and to provide pollen and nectar for bees. However, they also state that the nectar has a bitter taste that lowers the quality of the honey.

The recommended chemical control is Picloram applied at 0.4 to 0.6 kg/ha (Maddox 1982). Even though Picloram persists in the soil this treatment must be repeated after about 4 years to destroy the new plants germinating from the remaining seeds. Estimated costs of such treatment are $30 (Harris and Myers, 1984) and $37 (Maddox, 1982) per hectare. Since knapweed infestations occur extensively on land of low economic value, this high treatment cost often precludes the use of any chemical control whatsoever.

Therefore, biological control of diffuse knapweed has been investigated for use in the United States and Canada. To date, three insects have been established on this weed in North America. Two tephritid flies, Urophora affinis Frld. and U. quadrifasciata (Meig.), were released in Canada in 1970. Their larvae stimulate galls in the receptacle and reduce seed production. U. affinis was released in the western United States in 1973 (Maddox, 1979). In the spring of 1976, a root boring buprestid beetle, Sphenoptera jugoslavica Obenb., was released in British Columbia (Canada) followed by release in the United States in 1981. These insects were studied initially by Dr. Helmut Zwölfer at the Commonwealth Institute of Biological Control (CIBC) European Station in Delémont, Switwerland (Zwölfer 1970, Zwölfer 1976). All these insects are established in a number of locations in the United States and Canada. The effect of the gall flies on knapweed populations has been masked by recruitment from the seed bank in the soil. The effect of Sphenoptera has only recently been studied in detail (Harris and Myers 1984).

Despite the suitability of these established agents, additional natural enemies will be necessary in order to reduce diffuse knapweed to a sub-economic level with biological control.

The moth Pterolonche inspersa (Lepidoptera: Pterolonchidae), whose larvae mine the roots of diffuse knapweed, was screened by USDA Rome Laboratory (1980-84). In 1982 this insect was introduced into quarantine, at Albany, CA., for further testing.

A petition to release P. inspersa in the United States was recently (1986) submitted to and approved by the Technical Advisory Group for the Biological Control of Weeds, (TAG) USDA, Animal Health Inspection Service (APHIS), and releases were made in 1987.

Efforts to find additional biological control agents which could further reduce the density of diffuse knapweed led us to focus on the potential of Bangasternus fausti (Reitter) (Coleoptera: Curculionidae), a weevil whose larvae feed and develop in the flowerheads of Centaurea spp.. A heavy attack by this weevil significantly reduces the seed production of the infested plants.

Bionomical and host specificity studies of B. fausti, started at Rome (Italy) and Thessaloniki (Greece) laboratories (1985-86) are still underway. The results of these trials allow us to petition for the introduction of B. fausti into quarantine in the U.S. for more testing. If approval is granted, simultaneous testing will be conducted in Europe on the remaining European plants in the list and in the U.S. on native American plant species closely related to the target weed or on plants of economic importance.

Taxonomy

Bangasternus fausti whose adult was described by Reitter in 1890 is in the family Curculionidae, subfamily cleoninae and tribe lixini.

The genus Bangasternus Des Gozis, 1886 contains nine species (Table 1) which are distributed in the Palearctic region (Ter-Minasyan 1978).

Adults of B. fausti are about 4-5 mm. long (Fig.1). Basically this weevil is blackish color, with the whole body covered with thin white setae which confer a greyish color. Females are larger than males and can be differentiated from the males by the convex pygidium.

Geographic Distribution: According to Ter-Minasyan (1978) B. fausti is distributed in the Caucasus (Araks Valley) and Armenia. In a survey of the taxonomic litterature made by Dott. E. Colonnelli, (Curculionid specialist, University of Rome, Italy) on behalf of U.S.D.A. Rome Laboratory, he found that B. fausti is distributed in Romania, Bulgaria, Macedonia, Turkey, Armenia, Iran, Greece, and Italy. B. fausti was first found in Italy only recently on C. calcitrapa (1985). (Colonnelli personal communication).

Host Plants

B. fausti was recorded on Centaurea squarrosa in Armenia by Ter-Minasyan 1967 (1978). In addition we found this weevil on Centaurea diffusa, C. maculosa, C. calcitrapa and C. solstitialis in Northern Greece. There are no literature records of B. fausti as a pest of cultivated plants in Europe (Balachowsky 1963; Della Beffa 1961).

Mortality factors: Eggs and larvae of B. fausti are often parasitized in northern Greece. No figures are presently available on the percent parasitization. Specimens of some parasites have been sent to the USDA ARS Systematic Entomology Laboratory (SEL) for determination.

Studies of the larval mortality were conducted in quarantine, at the USDA Rome Laboratory, in June-July 1985. Three-hundred diffuse knapweed seedheads, on which females of B. fausti laid their eggs were followed until the formation of new adults. A sample of 10 infested seedheads were dissected at weekly intervals and the number of living and dead larvae were recorded. Another stock of infested seedheads (n=400) were left undisturbed until adult emergence.

Results

From the second stock (n=400) of infested seedheads, on which ca. 1000 eggs were found, 120 adults of B. fausti emerged in May 1986. This figure, assuming 99% of eggs were fertile, indicated that ca. 88% of the hatched larvae died during their development. Apparently, the following factors played an important role in limiting the development of the population of B. fausti larvae:

(a) Competition: If more than one egg was oviposited on the stem of C. diffusa plants, only one larva, through a gallery made in the stem, reached the inside of the seedhead where it completed its development. If the entrance hole in the stem had been made, the other larvae were found dead either on the stem or with a portion of the body in the stem. Neonate larvae hatched from eggs laid on the bracts mined directly the seedhead, but some larvae were found dead in the inner faces of the bracts.

(b) Seedhead size: Under laboratory conditions, it was noted that seedheads of 4-5 mm. diameter supported the complete development of B. fausti (fig.2). In smaller seedheads 2-3 mm. in diameter, the larvae did not develop.

## LIFE HISTORY

### Material and Methods

The biology of B. fausti was studied in two localities (Panorama and Thermi) near Thessaloniki (Northern Greece) in 1985-86. Observations on adult emergence, mating, and oviposition behavior were made at the end of April until July. In addition, data on the egg fertility, the pre-eclosion period, and observations on the behavior on neonate larvae were obtained under laboratory conditions.

The population density and the period of adult activity was determined at Panorama and Thermi from May 13-23, 1985. In each site, a sample of 100 diffuse knapweed plants was inspected for 10 days, early in the morning (7.00 - 9.00 AM), in the mid-day (12.00 - 14.00 PM) and at sunset. The number of weevils found on each plant was counted and recorded.

The field host-range of B. fausti was investigated by examining 23 plant species in the Compositae family, naturally growing together with C. diffusa in the following localities in Greece: Thermi, Panorama, and Arnea. For each site, a sample of 38-60 plants of each species was inspected, recording the number of adults of B. fausti.

### Results

B. fausti adults overwinter in the seedheads of C. diffusa. In 1985 adults of B. fausti first appeared in the field on May 7 (mean 5 adults/100 plants), peaked at the end of May (mean 275 adults/100 plants) and continued until the first decade of June. The first copulating adults in the field were seen on May 12.

The oviposition of B. fausti continues in the field until the end of July. The ovipositional behavior of five females was observed on plants of C. diffusa at Thermi on mid-June. Females moved from one branch to another searching for the appropriate bud, spending 20-30 minutes searching. Once the bud was selected (3.5-4 mm long; 3-3.5 mm. diam.), the female examined it carefully for 4-5 minutes to determine the oviposition site. Some of the light pubescence on the oviposition site was consumed as food, the remaining was cut-off and pushed aside by the female, leaving a smooth surface. The oviposition site was prepared in 5-10 minutes then the female positioned her abdomen against the seedhead bracts and commenced oviposition which lasted 3-4 minutes. Once the eggs were laid, they were covered with a dark green material which the females exuded from her anal end. In 5-10 minutes this material hardened into a blackish egg cover. In the field, eggs were either oviposited on the bracts of the seedheads in proximity of the receptacle or on the terminal portion of the stem. Eggs were usually laid individually on the bracts, but occasionally, two-three eggs were counted on the same bract, or stem terminal.

In the laboratory, (Temp. 15-30; RH. 30-70%; L/D 16/8 hrs), the eggs hatched in 8-12 days and were 97% fertile in a sample of 500 eggs. The neonate larvae of B. fausti mined into the stem and made a tunnel to the bud, where they completed the larval development almost completely consuming the seedhead content. Observations in the laboratory (Temp. 15-30; RH. 30-70%; L/D 16/8 hrs) suggested that B. fausti was able to complete its life cycle, from eggs to adult, in ca. 32 days.

Teneral adults of this weevil can be found in the field by dissecting mature diffuse knapweed seedheads in the second decade of July. From a sample of 200 seedheads of diffuse knapweed artificially infested in mid-June 1985 at the Rome Laboratory, only 15 new adults emerged at the end of July. The remaining adults emerged nine months later (end of April 1986) indicating that the newly formed adults hibernated in the seedheads until the following spring.

Forty percent of the C. diffusa plants inspected in Panorama and 24% of the plants in Thermi were infested with adults of B. fausti. The population density of the weevil was higher in Panorama ($\bar{x}$ 2.8 $\pm$ 4.8 adults/plant; range 1-20) than in Thermi ($\bar{x}$ 0.8 $\pm$ 2.0 adults/plant; range 1-14). No significant differences in density were evident between the morning, in the mid-day and at sunset examinations.

Examination of related thistles in the field indicated a narrow host-spectrum of B. fausti confined to plants in the genus Centaurea (i.e. C. diffusa, C. solstitialis, C. calcitrapa, and C. maculosa). These data are presented in table 1.

Effect of organism on host plant: To determine the effectiveness of B. fausti in reducing the seed production in infested heads of C. diffusa, samples of infested (n = 20) and uninfested heads (n = 20) were collected on June 30, 1986. The samples were dissected and the seeds found in each were collected and their number recorded. The collected seeds were sown in a peat based germination medium (JIFFY 7)[1/] and kept in the glasshouse for two months to measure the percent germination.

1/ Jiffy Products Ltd., Norway

From the healthy capitulae 240 seeds were collected (11-13 seeds/seedhead), while the infested heads (No. = 5) produced only five seeds ($\bar{x}$ 0.25 seeds/seedhead). None of the seeds from the infested seedheads germinated, while 90% of those from healthy seedheads germinated in 5-7 days. These data indicated that the seedhead content was almost destroyed by *B. fausti* larval feeding and that the seeds which were not eaten were no longer viable.

In the field, infested seedheads generally become dry earlier than the healthy ones, turn a pale brown color, and the top appears slightly open.

Potential control value

Using the system developed by Harris (1973) to estimate the value of a potential biological control agent, we arrived at a score of 20, the category of those agents having good prospects as biological control of weeds agents.

Effectiveness score of *B. fausti*:

| | | |
|---|---|---|
| 1. | Host specificity | 3 |
| 2. | Direct damage inflicted | 5 |
| 3. | Indirect damage inflicted | 0 |
| 4. | Phenology of attack | 2 |
| 5. | No. of generation | 0 |
| 6. | No. of progeny/generation | 0 |
| 7. | Extrinsic mortality factors | 0 |
| 8. | Feeding behavior | 0 |
| 9. | Compatibility | 2 |
| 10. | Distribution | 4 |
| 11. | Effectiveness | 4 |
| 12. | Size | 0 |
| | Total | 20 |

Host specificity experiments

Test plants: To determine the host plant range of B. fausti, host specificity testing (Larval survival, and oviposition tests) were conducted in 1985-86 using 30 plant species or varieties in the family compositae. The entire test plant list for B. fausti included ca. 65 test plants (Table 3). These test plants not yet tested will be included in the experiments which will be done in 1987-88.

## NO CHOICE OVIPOSITION TEST

Material and Methods

Adults of B. fausti were collected on C. diffusa near Thessaloniki (Northern Greece) in May-June, 1985-86. Each year ca. 1500 adults were collected. The adults were separated according to sex at the USDA, Thessaloniki Laboratory and then shipped to the USDA Laboratory at Rome. Prior to setting up the experiment, adults of B. fausti were fed on plants of diffuse knapweed for 3-5 days to allow them to recover from possible stress due to the trip. Later, these adults were placed on potted plants covered with transparent plastic cylinder cages (20 cm diam; height 70 cm) with four holes (10 cm diam) in the walls covered with nylon organdy. The top of the cylinder was capped with nylon organdy kept in place with a rubber band. On each potted plant 2 $^{oo}$ and 2 oo weevils were used. One potted plant served as a replicate. Test plants were inspected for eggs twice/week, and any eggs found were left undisturbed to permit larval development. At each inspection the feeding damage was also recorded.

In 1985, 14 test plants plus the control were included in the trial. All the test plants were replicated 10 times except for C. solstitialis (USA), C. americana (USA), Cirsium douglasii (USA), and Cynara scolymus which had fewer but not less than six replicates.

In 1986, 16 test plants plus the control were tested. All the test plants were replicated 10 times except for C. diffusa (Spokane, USA) and Onopordum acanthium which had seven and six replicates respectively. The plants C. solstitialis, C. americana, C. alba, C. pseudoalba and Cynara scolymus were tested in both 1985 and 1986 in the quarantine greenhouse with fluctuating temperature (rate 15°C - 30°C; and RH (range 30%-70%) and natural day length).

Results

B. fausti was able to oviposit only on plants of the genus Centaurea. Within this spectrum, eggs were found on the American biotypes of diffuse knapweed, on C. maculosa, C. alba and C. pseudoalba and C. nicaensis, as well as on the control. No eggs were laid on C. americana and on American biotypes of C. solstitialis. Results are summarized in Tables 4 and 4A.

Choice oviposition test

Material and methods: The objective of this trial was to determine if adults of B. fausti, in presence of its natural host (C. diffusa), would oviposit on the exposed test plants. The insects tested in this experiment came from the same stock of adults used in the former trial, which were collected in 1986. The experiment consisted in placing together, in the same pot (32 cm. diam.), 2 test plants and the control and confining them with B. fausti (4 ♀♀, 4 ♂♂) in a transparent cylinder cage, already described. One pot represented a replicate, and the experiment was replicated twice. The plants were inspected twice/week until all the females were dead.

This experiment was conducted in the quarantine greenhouse from mid-June to first ten days of August (Temp. 15°-30°C, RH 30-70%, natural light).

Results

The data obtained in this trial followed the same pattern of the previous experiment. Eggs were found on C. maculosa, C. alba and C. pseudoalba. No eggs were laid on C. nicaensis, (on which 5 eggs were found in the preceding trial) nor on the American biotype of C. solstitialis. Results are presented in Table 5.

## LARVAL SURVIVAL TEST

Material and Methods

Neonate larvae of B. fausti are very delicate and difficult to manipulate without damage, so mature fertile eggs were used for this trial. The eggs were produced in laboratory using adults of B. fausti collected on C. diffusa in Northern Greece in 1985-86. To determine the spectrum of plants which support larval development, eggs were transferred to the test plants with a fine camel brush, inserting them between the bracts of the seedheads. On each test plant, 30 eggs of B. fausti were distributed over 15 seedheads (2 eggs/seedhead). One seedhead represented a replicate. The infested seedheads were marked and inspected daily to see if the eggs had hatched.

All the infested seedheads were dissected ca. 30 days after the beginning of the experiment, the time required for B. fausti to complete its development on C. diffusa. The experiment started June 25 and ended July 27. In 1985, 14 test plants and the control were tested with 15 replicates, except for C. americana and C. solstitialis which were replicated nine and ten times respectively. The test was done in the quarantine greenhouse under the same conditions as preceding trials.

Results

Neonate larvae of B. fausti, completed development only on the American biotypes of diffuse knapweed, on C. maculosa and the control plants. Results are summarized in Table 6-6A.

## DISCUSSION

Data gathered during the host specificity tests on B. fausti showed that host suitability for a plant species could be determined in the first 10 days of the experiments, because adults and larvae demonstrated more oviposition and feeding activity during that time. If within the first 10 days neither oviposition nor feeding occurred on the exposed test plants then they would not be attacked during the rest of the experiments.

Different factors may determine host plant selection by phytophagous insects: presence or absence of attractants or repellents, presence or absence of feeding stimulants, and mechanical barriers due to the plant's morphology. Our host specificity tests with B. fausti showed a very restricted spectrum of suitable hosts and no potential adaptability to non-host plants. Other than the Centaurea diffusa control originating from Greece, and the closely related American biotypes of diffuse and spotted knapweed originating from Washington, Idaho, and Montana; oviposition also occurred on C. alba, C. pseudoalba and C. nicaeansis. Although these three plants have the necessary requisites to induce this weevil to feed and oviposit, none of the neonate larvae were able to bore into plant tissue because of the hard bracts of these three European Centaurea spp. Two other interesting observations of natural barriers that in some way avoid insect attack were noted. For example, many living adults were seen entrapped in the long hair of the weed Carthamus lanatus, unable to free themselves. The seedhead of Helianthus annus, if cut, produces a viscous substance that entraps and kills first-instar larvae of B. fausti. Not all the unsuitable test plants possessed these natural defense barriers, (at least not visible to

the researcher) but considering the total failure of this weevil to oviposit and survive on non-host plants, other occult defense mechanisms were no doubt working. In our opinion, our host specificity findings are sufficient to classify B. fausti as an effective, specific biocontrol agent of diffuse and spotted knapweeds and make it a valid candidate for introduction into the United States for testing of indigenous plants in quarantine and most probably release at a later time.

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Zwölfer, H. 1976. Investigations on Sphenoptera (Chilostetha) jugoslavica Obenb. (Col. Buprestidae), a possible biocontrol agent of the weed Centaurea diffusa Lam. (Compositae) in Canada. Zeit. ang. Entomol., 80: 170-190.

TABLE 1

Tabular summary of biological information on *Bangasternus*.

| Species of *Bangasternus* | Known Geographic distribution | Recorded Host Plants |
|---|---|---|
| *araxis* Reitter | Caucasus Mountains and Turkestan (USSR); Central Asia | unknown |
| *diecki* Capiomont | Southern Spain | unknown |
| *fausti* Reitter | Araxestal (Caucasus) USSR; Armenia | *Centaurea diffusa*, *C.maculosa*, *C.calcitrapa* *C.solstitialis* 1/, *C.squarrosa* |
| *orientalis* Capiomont *smyrnensis* Cap.3/ | Southeast Europe including Austria; Balkans; Asia Minor including Turkey; Caucasus Mountains and Turkestan (USSR); Israel; Lebanon. | *Centaurea solstitialis*, *C.iberica* |
| *planifrons* Brulle' | Eastern Mediterranean; Turkmenia; Greece; Asia Minor; Syria | *Centaurea calcitrapa* 2/ |
| *provincialis* Fairmaire | France; Italy | *Centaurea nigra*. *C.paniculata*, *C.scabiosa* |
| *siculus* Capiomont | Sicily; Spain | unknown |
| *syriacus* Stierlin | Syria | unknown |
| *villosus* Capiomont *hispanicus* Cap.3/ | Spain; Morocco | unknown |

1/ These records are a result of the research discussed in this petition.

2/ *C. calcitrapa* is another new host record.

3/ Subspecies.

Field Host Range of Bangasternus fausti R. at three localities in Northern Greece (Thessaloniki) 1985.

| Location | Plant species cecked | sample size | N° Adults found |
|---|---|---|---|
| THERMI | Centaurea diffusa (control) | 60 | 48 |
| | Centaurea solstitialis | 50 | 7 |
| | Centaurea calcitrapa | 48 | 4 |
| | Centaurea maculosa | 50 | 7 |
| | Centaurea salonitana | 38 | 0 |
| | Centaurea macedonica | 38 | 0 |
| | Centaurea scabiosa | 39 | 0 |
| | Carduus nutans | 50 | 0 |
| | Carduus pycnocephalus | 55 | 0 |
| | Carduus candicans | 40 | 0 |
| | Onopordum acanthium | 45 | 0 |
| | Onopordum illyricum | 50 | 0 |
| | Cirsium lanceolatum | 39 | 0 |
| | Cirsium arvense | 42 | 0 |
| | Carthamus dentatus | 38 | 0 |
| | Carthamus lanatus | 42 | 0 |
| | Silybum marianum | 50 | 0 |
| | Carlina corymbosa | 39 | 0 |
| | Carlina acaulis | 41 | 0 |
| | Galactites tomentosa | 50 | 0 |
| | Cnicus benedictus | 39 | 0 |
| | Echinops sphaerocephalus | 42 | 0 |
| | Sonchus oleraceous | 38 | 0 |
| | Cynara scolymus 1/ | 50 | 0 |
| PANORAMA | Centaurea diffusa (control) | 60 | 65 |
| | Centaurea solstitialis | 39 | 2 |
| | Centaurea calcitrapa | 42 | 1 |
| | Carduus nutans | 39 | 0 |
| | Carduus pycnocephalus | 43 | 0 |
| | Onopordum acanthium | 50 | 0 |
| | Cirsium lanceolatum | 43 | 0 |
| | Cirsium arvense | 41 | 0 |
| | Carthamus lanatus | 43 | 0 |
| | Silybum marianum | 50 | 0 |
| | Carlina corymbosa | 38 | 0 |
| | Galactites tomentosa | 50 | 0 |
| | Cnicus benedictus | 39 | 0 |
| | Echinops sp. | 50 | 0 |
| | Sonchus sp. | 39 | 0 |
| ARNEA | Centaurea diffusa (control) | 60 | 42 |
| | Centaurea maculosa | 39 | 3 |
| | Centaurea calcitrapa | 45 | 2 |
| | Carduus nutans | 50 | 0 |
| | Carduus pycnocephalus | 50 | 0 |
| | Cirsium arvense | 60 | 0 |
| | Cirsium lanceolatum | 39 | 0 |
| | Cirsium spinosissimum | 45 | 0 |
| | Carthamus lanatus | 53 | 0 |
| | Carthamus dentatus | 38 | 0 |
| | Carlina acaulis | 41 | 0 |
| | Carlina corymbosa | 50 | 0 |
| | Galactites tomentosa | 60 | 0 |
| | Cynara scolymus 2/ | 43 | 0 |

1/- 2/ Private gardens of Cynara scolymus were cecked for the presence of B. fausti.

TABLE 3

page 1. SPECTRUM OF TEST PLANTS PROPOSED FOR TESTING *Bangasternus*

| Test Plant | *Bangasternus fausti* (DKW seed feeder) |
|---|---|
| RANALES | |
| Ranuncolaceae | |
| *Ranunculus asiaticus* L. | 0 |
| *Ranunculus auricomus* L. | NYT (OI) |
| RHOEDALES | |
| Papaveraceae | |
| *Papaver somniferum* L. | + (CH) |
| Cruciferae | |
| *Brassica oleracea* L. | 0 |
| CENTROSPERMAE | |
| Caryophyllaceae | |
| *Silene vulgaris* (Moench) | NYT (CH) |
| *Silene nutans* L. | 0 |
| *Silene armeria* L. | 0 |
| Amaranthaceae | |
| *Iresine Lindenii* (Van Houtte) | 0 |
| MALVALES | |
| Malvaceae | |
| *Malva alcea* L. | NYT (CH) |
| VIOLALES | |
| Violaceae | |
| *Viola tricolor* L | NYT (OI,CH) |
| ROSALES | |
| Leguminosae | |
| *Medicago sativa* L. | 0 |
| GERANIALES | |
| Euphorbiaceae | |
| *Euphorbia lathyris* L. | 0 |
| *Euphorbia polychroma* K. | 0 |
| UMBELLIFLORAE | |
| Umbelliferae | |
| *Foeniculum vulgare* Miller (= *F. officinale* All.) | 0 |
| *Apium graveolens* L. | 0 |

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| Test Plant | Bangasternus fausti (DKW seed feeder) |
|---|---|
| TUBIFLORAE | |
| Labiatae | |
| Mentha sp. | 0 |
| Scrophulariaceae | |
| Antirrhinum majus L. | NYT (OI,CH) |
| Linaria dalmatica Mill. | 0 |
| PLANTAGINALES | |
| Plantaginaceae | |
| Plantago lanceolata L. | 0 |
| DIPSACALES | |
| Dipsacaceae | |
| Scabiosa caucasica Bieb. | 0 |
| CAMPANULALES | |
| Compositae | |
| Subfamily ASTEROIDIDAE | |
| - Tribe Heliantheae | |
| Helianthus annuus (L.) | + (AI,CH,RG) |
| Helianthus tuberosus (L.) | NYT (OI,CH,RG) |
| Zinnia elegans Jacq. | + (OI,CH,RG) |
| Rudbeckia hirta L. (*) | NYT (OI,CH,RG) |
| - Tribe Astereae | |
| Aster chinensis (Nees.) | NYT (OI,CH,RG) |
| - Tribe Calendulae | |
| Calendula officinalis (L.) | NYT (OI,CH,RG) |
| - Tribe Anthemidae | |
| Chrysanthemum leucanthemum L. | + (OI,CH) |
| Achillea millefolium (L.) | + (OI,CH,RG) |
| Artemisia dracunculus L. | 0 |
| Artemisia vulgaris L. | 0 |
| Tanacetum vulgare L. | NYT (CH,RG) |
| Tanacetum parthenium(L.)Sch.-Bip. | NYT (AI,RG) |
| - Tribe Senecioneae | |
| Senecio jacobaea L. | 0 |
| - Tribe Heleniae | |
| Tagetes erecta Hort. | NYT (OI,CH,RG) |
| - Tribe Arctotidae | |
| Gazania splendens E.G.& Henderson | + (OI,CH) |
| - Tribe Carduae | |
| Arctium minus Schk. | NYT (CH,RG) |
| Carduus nutans L. | 0 |
| Carduus acanthoides L. | 0 |
| Carduus pycnocephalus Jacq. | 0 |
| Carduus tenuiflorus Curt. | 0 |
| Carduus thoermeri Weinman | NYT (CH,RG) |

page 3

| Test Plant | | Bangasternus fausti (DKW seed feeder) |
|---|---|---|
| Cirsium lanceolatum L. (Scop.) | | NYT (CH,RG) |
| Cirsium palustre L. (Scop.) | | NYT (CH,RG) |
| Cirsium arvense L. | | + (CH,RG) |
| Cirsium eriophorum L. (Scop.) | | NYT (CH,RG) |
| Cirsium discolor (Muhl) | (*) | + (CH,RG) |
| Cirsium occidentale (Nutt) | (*) | NYT (CH,RG) |
| Cirsium undulatum (Nutt) | (*) | NYT (CH,RG) |
| Cirsium douglasii Jepson | (*) | NYT (CH,RG) |
| Cirsium andrewsii (Gray) | (*) | NYT (CH,RG) |
| Cirsium flodmani (Rydb) | (*) | NYT (CH,RG) |
| Cirsium pitcheri (Torr) | (*) | NYT (CH,RG) |
| Cirsium hillii (Canby) | (*) | NYT (CH,RG) |
| Onopordum acanthium L. | | + (CH,RG) |
| Cynara scolymus L. | | + (AI,CH,RG) |
| Silybum marianum (Gaemtn) | | + (CH,RG) |
| Saussurea americana (D.C.) | | NYT (RG) |
| Serratula radiata (Waldst) | | 0 |
| Serratula nudicaulis (L.)DC. | | NYT (OI,CH,RG) |
| Galactites tomentosa Moench | | NYT (CH,RG) |
| Carlina acaulis L. | | 0 |
| Carlina corymbosa L. | | NYT (CH,RG) |
| Cousinia sp. Cass. | | NYT (CH,RG) |
| Echinops ritro L. | | NYT (RG) |
| Cnicus benedictus L. | | + (CH,RG) |
| Carthamus tinctorius L. | | + (AI,CH,RG) |
| Carthamus lanatus L. | | + (CH,RG) |
| Carthamus dentatus L. | | 0 |
| - Subtribe Centaureinae | | |
| - Subgenus Lopholoma | | |
| Section Lopholoma | | |
| Centaurea scabiosa L. | | + (CH,RS) |
| - Subgenus Acrolophus | | |
| Section Pannophyllum | | |
| Centaurea cineraria L. | | NYT (OI,CH,RS) |
| Centaurea friderici Vis. | | NYT (CH,RS) |
| Centaurea crithmifolia Vis. | | 0 |
| Section Paniculatae | | |
| Centaurea paniculata L. | | NYT (CH,RS) |
| Section Maculosae | | |
| Centaurea maculosa Lam. | | + (CH,RS) |
| Centaurea rhenana Bor. | | NYT (CH,RS) |
| Section Cylindracea | | |
| Centaurea diffusa Lam. | | + (HP) |
| - Subgenus Calcitrapa | | |
| Centaurea calcitrapa L. | | + (CH,RS) |
| - Subgenus Seridia | | |
| Centaurea napifolia L. | | NYT (CH,RS) |

TABLE 3 contd.

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| Test Plant | | Bangasternus fausti (DKW seed feeder) |
|---|---|---|
| - Subgenus Solstitiaria | | |
| Centaurea sostitialis L. | | + (CH,RS) |
| Centaurea nicaeensis All. | | + (CH,RS) |
| - Subgenus Phalolepis | | |
| Section Phalolepis | | |
| Centaurea alba L. | | + (CH,RS) |
| Centaurea pseudoalba L. | | + (CH,RS) |
| - Subgenus Jacea | | |
| Section Jacea | | |
| Centaurea jacea L. | | NYT (CH,RS) |
| - Subgenus Cyanus | | |
| Centaurea cyanus L. | | + (CH,RS) |
| Centaurea axillaris Willd. | | NYT (CH,RS) |
| Section Arenariae | | |
| Centaurea cristata Brtt | | NYT (CH,RS) |
| Unassigned Section | | |
| Centaurea carsiana (Scop.) | | NYT (CH,RS) |
| Centaurea americana | (*) | + (CH,RS) |
| Subfamily CICHORIOIDEAE | | |
| Scolymus hispanicus L. | | NYT (CH,RG) |
| Cichorium intybus L. | | + (AI,RG) |
| Lactuca sativa L. | | NYT (AI,RG) |

1/ In parentheses: reason(s) for testing.
0 = Species not proposed as a test plant.
+ = Species tested.
NYT = Species not yet tested.
CH = Capitulum capable of hosting insect.
CNH = Capitulum not capable of hosting insect.
OI = Ornamental importance.
AI = Agricultural importance.
(*) = American native species.
PNS = Plant species not synchronized with insect emergence.
HP = Host plant.
RG = Related genus.
RS = Related species.
RCH = Root capable of hosting insect.
RNH = Root not capable of hosting insect.

N.B.: Plant taxonomy according to :
- "Flora Europaea" edited by Tutin, Tg., et al., Cambridge University Press, 1976.

Economic importance according to:
- Polunin O., "Flowers of Europe", edited by Oxford University Press, 1969.
- Pignatti S., "Flora d'Italia", edited by Edagricole, 1982.
- Bailey L., "Manual of Cultivated Plants", edited by The MacMillan Company, 1960.

Results of oviposition no choice test of Bangasternus fausti R.
Rome, Italy, 1985.

| Test plants | Total N°of replicates | Total N°of insects in replicates ♀♀ | ♂♂ | N°seed heads exposed/rep. X̄ ± S.D | N°seed heads infested/rep. X̄ ± S.D | %of seed heads infested/rep. X̄ ± S.D | N°eggs ovoposited in replicates X̄ ± S.D |
|---|---|---|---|---|---|---|---|
| Centaurea diffusa (control) | 10 | 20 | 20 | 156.7 33.5 | 102 12.5 | 71.9 22.5 | 213 89 |
| Centaurea alba | 10 | 20 | 20 | 39.6 11.1 | 19 5.3 | 43.3 30.3 | 58.8 5 |
| Centaurea cyanus | 10 | 20 | 20 | 71.5 13.8 | 0 0 | 0 0 | 0 0 |
| Centaurea calcitrapa | 10 | 20 | 20 | 33.4 8.3 | 0 0 | 0 0 | 0 0 |
| Centaurea solstitialis (USA) | 5 | 10 | 10 | 15.8 4.3 | 0 0 | 0 0 | 0 0 |
| Centaurea americana (USA) | 1 | 2 | 2 | 0 0 | 0 0 | 0 0 | 0 0 |
| Cirsium arvense | 10 | 20 | 20 | 12.5 2.5 | 0 0 | 0 0 | 0 0 |
| Cirsium douglasii (USA) | 1 | 2 | 2 | 0 0 | 0 0 | 0 0 | 0 0 |
| Cnicus benedictus | 10 | 20 | 20 | 6.4 1.9 | 0 0 | 0 0 | 0 0 |
| Carthamus lanatus | 10 | 20 | 20 | 15.3 2.1 | 0 0 | 0 0 | 0 0 |
| Carthamus tinctorius (USA) | 10 | 20 | 20 | 14.5 2.5 | 0 0 | 0 0 | 0 0 |
| Cynara scolymus (USA) | 6 | 12 | 12 | 2.5 0.7 | 0 0 | 0 0 | 0 0 |
| Helianthus annuus (USA) | 10 | 20 | 20 | 2.8 1.1 | 0 0 | 0 0 | 0 0 |
| Zinnia elegans | 10 | 20 | 20 | 5.8 1.8 | 0 0 | 0 0 | 0 0 |
| Chrysanthemum leucanthemum | 10 | 20 | 20 | 16.9 2.8 | 0 0 | 0 0 | 0 0 |

TABLE 4A

Results of oviposition no choice test of Bangasternus fausti R.
Rome, Italy, 1986.

| Test plants | Total N°of replicates | Total N°of insects in replicates ♀♀ | ♂♂ | N°seed heads exposed/rep. X̄ ± S.D | N°seed heads infested/rep. X̄ ± S.D | %of seed heads infested/rep. X̄ ± S.D | N°eggs ovoposited in replicates X̄ ± S.D |
|---|---|---|---|---|---|---|---|
| Centaurea diffusa (control) | 10 | 20 | 20 | 98.8 43.9 | 56.5 33.5 | 54.3 25.7 | 94.5 73.3 |
| Centaurea diffusa (Spokane,USA) | 7 | 14 | 14 | 61.5 36.9 | 27.9 35.9 | 33.8 30.1 | 36.2 46.7 |
| Centaurea diffusa (Idaho,USA) | 10 | 20 | 20 | 98.2 69.6 | 39.6 26.1 | 39.2 23.3 | 52.1 33.0 |
| Centaurea maculosa (Montana,USA) | 10 | 20 | 20 | 58.1 13.1 | 25.3 13.5 | 43.8 19.8 | 68.6 51.1 |
| Centaurea americana (USA) | 10 | 20 | 20 | 1.6 0.6 | 0 0 | 0 0 | 0 0 |
| Centaurea alba | 10 | 20 | 20 | 17.7 9.5 | 14.1 11.2 | 70.3 33.5 | 48.2 42.5 |
| Centaurea pseudoalba | 10 | 20 | 20 | 28.0 12.1 | 21.7 11.5 | 74.8 9.8 | 77.5 47.0 |
| Centaurea nicaensis | 10 | 20 | 20 | 10.0 3.7 | 0.3 0.9 | 3.3 10.4 | 0.5 1,5 |
| Centaurea solstitialis (CA,USA) | 10 | 20 | 20 | 24.2 9.3 | 0 0 | 0 0 | 0 0 |
| Caurduus pycnocephalus | 10 | 20 | 20 | 5.3 2.1 | 0 0 | 0 0 | 0 0 |
| Onopordum acanthium | 6 | 12 | 12 | 4.8 1.9 | 0 0 | 0 0 | 0 0 |
| Cichorium intybus(USA) | 10 | 20 | 20 | 49.1 28.0 | 0 0 | 0 0 | 0 0 |
| Carthamus tinctorius (USA) | 10 | 20 | 20 | 6.1 1.9 | 0 0 | 0 0 | 0 0 |
| Cynara scolymus(USA) | 10 | 20 | 20 | 1.2 0.4 | 0 0 | 0 0 | 0 0 |
| Achillea millefolium | 10 | 20 | 20 | 185.6 73.4 | 0 0 | 0 0 | 0 0 |
| Gazania spendens | 10 | 20 | 20 | 6.6 1.5 | 0 0 | 0 0 | 0 0 |
| Papaver somniferum | 10 | 20 | 20 | 3.6 0.8 | 0 0 | 0 0 | 0 0 |

TABLE 5

Results of oviposition choice test of Bangasternus fausti R. Rome, Italy, 1986.

| TEST PLANT | N° REPLICATES | INSECTS IN EACH REPLICATE ♀♀ | ♂♂ | N°EXPOSED SEED HEADS $\bar{X} \pm$ S.D | | %OF INFESTED SEED HEADS $\bar{X} \pm$ S.D | | N°EGGS OVIPOSITED IN REPLICATE $\bar{X} \pm$ S.D | |
|---|---|---|---|---|---|---|---|---|---|
| CENTAUREA DIFFUSA (CONTROL) | 2 | 4 | 4 | 44.5 | I2.0 | I8.5 | 4.9 | I7.5 | I0.6 |
| CENTAUREA MACULOSA (MONTANA) | 2 | 4 | 4 | 249.0 | I9.7 | 45.0 | I.4 | I60.I | I6.9 |
| CARDUUS PYCNOCEPHALUS (ITALY) | 2 | 4 | 4 | 27.0 | 25.4 | 0 | 0 | 0 | 0 |
| CENTAUREA DIFFUSA (CONTROL) | 2 | 4 | 4 | 38.0 | I5.5 | 27.5 | 4.9 | I00.I | I3.2 |
| CENTAUREA ALBA (ITALY) | 2 | 4 | 4 | 40.5 | I6.2 | 24.5 | I3.4 | I0.0 | 2.8 |
| CICHORIUM INTYBUS (ITALY) | 2 | 4 | 4 | I84.5 | I43.5 | 0 | 0 | 0 | 0 |
| CENTAUREA DIFFUSA (CONTROL) | 2 | 4 | 4 | I29.0 | I2.7 | 58.5 | I0.6 | I28.0 | 4.2 |
| CENTAUREA SOLSTITIALIS (U.S.A.) | 2 | 4 | 4 | 45.0 | I6.9 | 0 | 0 | 0 | 0 |
| ACHILLEA MILLEFOLIUM (ITALY) | 2 | 4 | 4 | I59.0 | 38.I | 0 | 0 | 0 | 0 |
| CENTAUREA DIFFUSA (CONTROL) | 2 | 4 | 4 | 86.5 | 40.8 | 46.0 | I.4 | I28.0 | I4.0 |
| CENTAUREA PSEUDOALBA (ITALY) | 2 | 4 | 4 | 3I.0 | 22.6 | 22.0 | 7.I | 7.5 | 3.5 |
| CARTHAMUS TINCTORIUS (U.S.A.) | 2 | 4 | 4 | 6,0 | 2.8 | 0 | 0 | 0 | 0 |
| CENTAUREA DIFFUSA (CONTROL) | 2 | 4 | 4 | 83.5 | 35.0 | 27.5 | 2.5 | 52.0 | I.4 |
| CENTAUREA NICAENSIS (ITALY) | 2 | 4 | 4 | I7.0 | 7.I | 0 | 0 | 0 | 0 |
| PAPAVER SOMNIFERUM (ITALY) | 2 | 4 | 4 | 5.5 | 2.I | 0 | 0 | 0 | 0 |

TABLE 6

First instar larval survival test of Bangasternus fausti R.
Rome, Italy, 1985.

| Plants Tested | N°. replications | Total eggs used | Total eggs hatched | Feeding | Max development stage reached instars 1st | 2nd | 3rd | Pupa | adult |
|---|---|---|---|---|---|---|---|---|---|
| Centaurea diffusa (control) | 15 | 30 | 22 | Heavy | 0 | 0 | 8 | 5 | 2 |
| Centaurea alba | 15 | 30 | 27 | Ligth | 21 | 0 | 0 | 0 | 0 |
| Centaurea cyanus | 15 | 30 | 26 | Ligth | 19 | 0 | 0 | 0 | 0 |
| Centaurea calcitrapa | 13 | 26 | 24 | None | 24 | 0 | 0 | 0 | 0 |
| Centaurea solstitialis (USA) | 15 | 30 | 29 | None | 23 | 0 | 0 | 0 | 0 |
| Centaurea americana (USA) | 1 | 2 | 2 | None | 2 | 0 | 0 | 0 | 0 |
| Cirsium arvense | 15 | 30 | 22 | None | 18 | 0 | 0 | 0 | 0 |
| Cirsium douglasii (USA) | 2 | 4 | 4 | None | 3 | 0 | 0 | 0 | 0 |
| Cnicus benedictus | 15 | 30 | 25 | None | 21 | 0 | 0 | 0 | 0 |
| Carthamus lanatus | 15 | 30 | 25 | None | 23 | 0 | 0 | 0 | 0 |
| Carthamus tinctorius (USA) | 15 | 30 | 25 | None | 23 | 0 | 0 | 0 | 0 |
| Cynara scolymus (USA) | 7 | 14 | 14 | None | 13 | 0 | 0 | 0 | 0 |
| Chrisanthemum leucanthemum | 15 | 30 | 22 | None | 16 | 0 | 0 | 0 | 0 |
| Zinnia elegans | 15 | 30 | 22 | None | 17 | 0 | 0 | 0 | 0 |
| Helianthus annus (USA) | 15 | 30 | 27 | None | 25 | 0 | 0 | 0 | 0 |

a/ All 1st instar larvae found on test plants were dead; b/ 3rd instar larvae, pupae, and adults found on the controls were alive and very active.

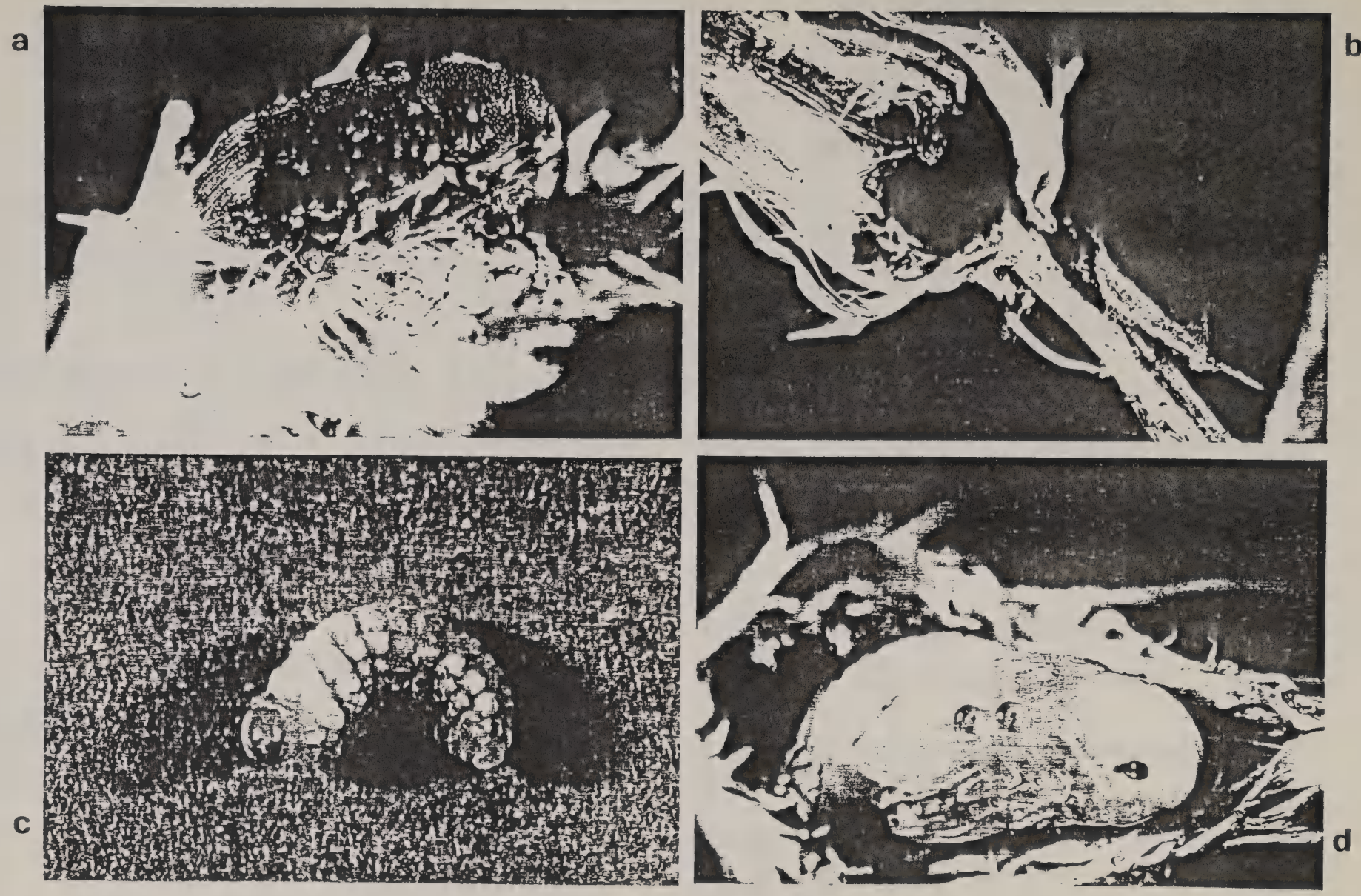
TABLE 6A

First instar larval survival test of Bangasternus fausti R.
Rome, Italy, 1986.

| Plants Tested | N°. replications | Total eggs used | Total eggs hatched | Feeding | Max development stage reached instars 1st | 2nd | 3rd | Pupa | adult |
|---|---|---|---|---|---|---|---|---|---|
| Centaurea diffusa (control) | 15 | 30 | 27 | Heavy | 10 | 0 | 10 | 2 | 3 |
| Centaurea diffusa (ID,USA) | 15 | 30 | 28 | Heavy | 11 | 0 | 7 | 5 | 1 |
| Centaurea diffusa (WAS,USA) | 15 | 30 | 25 | Heavy | 8 | 0 | 9 | 4 | 2 |
| Centaurea maculosa (Mont,USA) | 15 | 30 | 27 | Heavy | 9 | 0 | 10 | 2 | 2 |
| Centaurea americana (USA) | 9 | 18 | 18 | None | 12 | 0 | 0 | 0 | 0 |
| Centaurea alba (Italy) | 15 | 30 | 25 | None | 17 | 0 | 0 | 0 | 0 |
| Centaurea pseudoalba (Italy) | 15 | 30 | 27 | None | 21 | 0 | 0 | 0 | 0 |
| Centaurea solstitialis(Italy) | 10 | 20 | 19 | None | 15 | 0 | 0 | 0 | 0 |
| Centaurea nicaensis (Italy) | 15 | 30 | 28 | None | 20 | 0 | 0 | 0 | 0 |
| Achillea millefolium (Italy) | 15 | 30 | 26 | None | 18 | 0 | 0 | 0 | 0 |
| Carduus pycnocephalus (Italy) | 15 | 30 | 30 | None | 19 | 0 | 0 | 0 | 0 |
| Cichorium intybus (USA) | 15 | 30 | 28 | None | 12 | 0 | 0 | 0 | 0 |
| Carthamus tinctorius (USA) | 15 | 30 | 30 | None | 16 | 0 | 0 | 0 | 0 |
| Lactuca sativa (USA) | 15 | 30 | 28 | None | 21 | 0 | 0 | 0 | 0 |
| Cynara scolymus (USA) | 15 | 30 | 30 | None | 22 | 0 | 0 | 0 | 0 |

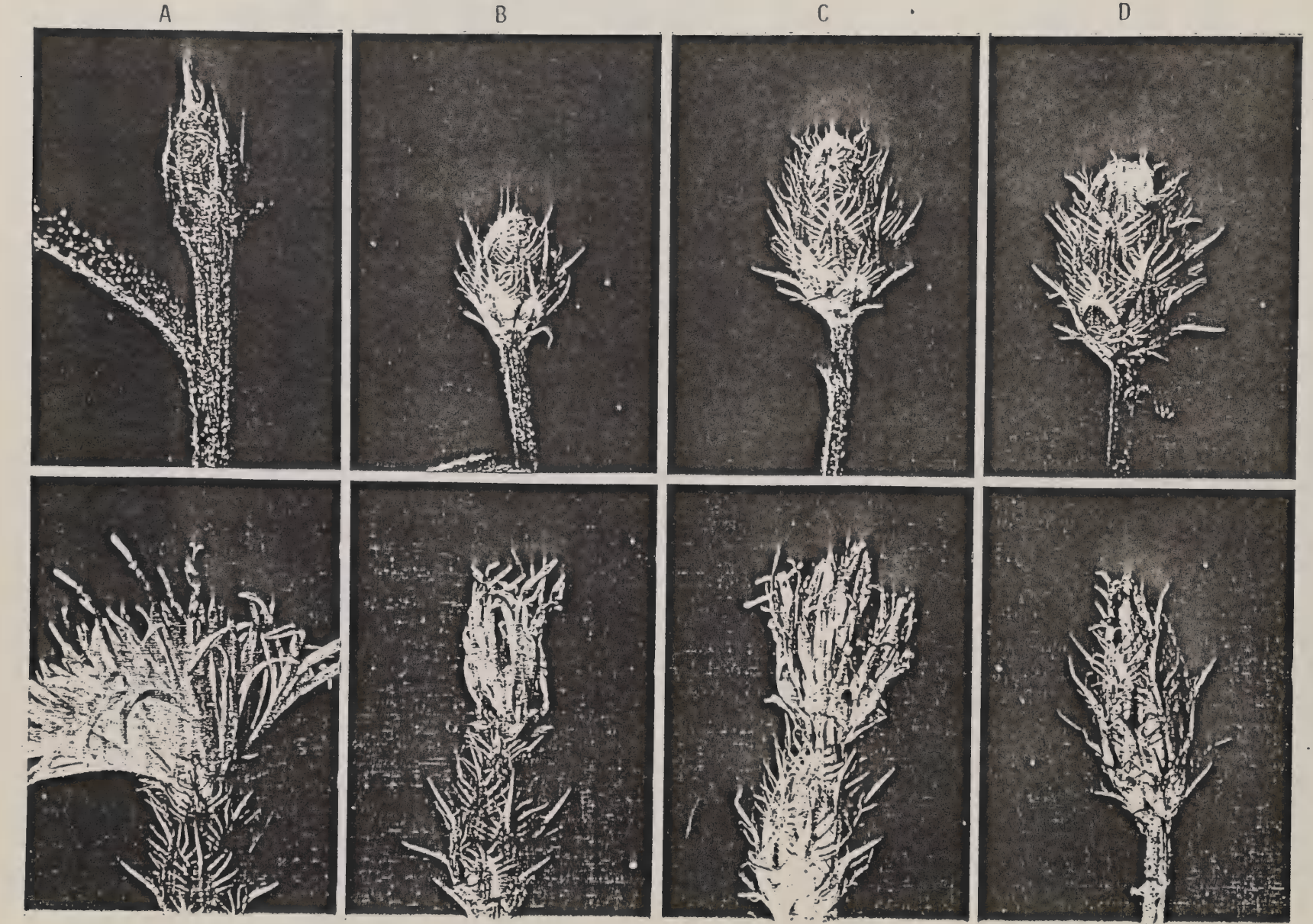
a/ All 1st instar larvae found on test plants were dead; b/ 3rd instar larvae, pupae, and adults found on the controls were alive and very active.

FIGURE 1




Bangasternus fausti Reitter (Col. Curculionidae): a) adult, b) larval damage, c) mature larva, d) pupa.

FIGURE 2




Buds and flower stages of Centaurea diffusa Lam

Photo C. Seed-head size able to support B. fausti development

## Miscellaneous Collections for ARS and CIBC

### ITALY

A collecting trip (seven days) was made in Southern Italy to collect Centaurea capitulae infested with the trypetid flies Orellia sp. and Terellia sp. About two thousand infested seedheads of Centaurea alba were collected in fields around Bari. These flowerheads were brought from Bari to the Rome laboratory, where they were cut from the branches and shipped to CIBC, Delémont, Switzerland. Other plants in the genus Centaurea, difficult to get from Botanical gardens, were also collected at Bari to use in the host specificity tests of B. fausti. About 50 rosettes of Centaurea alba, C. pseudoalba, and C. dealbata were dug up and brought back to the Rome Laboratory, where they were transplated in 22-diameter pots for use in host specificity trials.

USDA-ARS, Thermi (Thessaloniki), GREECE.

Rouhollah Sobhian

The year 1986 was devoted mostly to collections. Thistle heads were collected in June and August, and Bangasternus orientalis, Urophora sirunaseva, Chaetorellia hexachaeta, Eustenopus hirtus, Simyra dentinosa, Aceria centaurea, Aceria convolvuli, Chrysolina etc. were collected in May and June. About 2,500 kilometers were driven in Greece (see list of shipments) to make these collections. In addition, the study on the longevity and fecundity of Bangasternus fausti (a candidate for biological control of Centaurea diffusa) was completed and there was no time for doing other research projects.

Centaurea diffusa project

a) Longevity and fecundity of Bangasternus fausti

An experiment was designed to determine the longevity, fecundity, and the effect of the presence of males on copulation and oviposition. As soon B. fausti could be found on C. diffusa plants at Thermi (May 27 and 28, 1986), 18 copulating pairs were collected. Nine pairs were caged on C. diffusa bouquets (one pair/cage) (Group A). Of the other 9 pairs, only the females were caged on C. diffusa bouquets, one copulated female/cage (group B). The experiment started on May 28 and lasted until September 3, 1986. The bouquets were kept in vials with water and were replaced daily. The number of eggs laid by each female was recorded, and egg hatch was checked 9 days later. The eggs laid by groups A and B were kept separate to determine how long after the last copulation the females would produce fertile eggs. One liter trasparent plastic containers, with two holes (5 cm. diameter), covered with screen cloth, were used as cages. The cages were kept in the laboratory, near a window which provided sufficient light.

The results of the experiment are summarized in Table 1.

Table 1. Longevity and fecundity of Bangasternus fausti under laboratory conditions. Group A (♂ x ♀) and Groub B (single copulated ♀)

GROUP A (♀ x ♂)

| | Replicate No. | | | | | | | | | Mean/ replicate |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | |
| No. eggs/female | 359 | 6 | 0 | 26 | 2 | 0 | 32 | 180 | 0 | 67.2±116.6 |
| Mean eggs/°/day | 6.8±3.6 | 0.3±0.6 | 0 | 0.7±1.4 | 0.7±0.3 | 0 | 1.1±2.40 | 3.4±3.6 | 0 | 1.4±2.3 |
| Max No. eggs °/day | 15 | 2 | 0 | 6 | 1 | 0 | 8 | 11 | 0 | - |
| No. days °° laid eggs | 53 | 5 | 0 | 25 | 20 | 0 | 7 | 39 | 0 | 16.5±18.1 |
| No. days °° lived | 53 | 18 | 8 | 40 | 30 | 46 | 29 | 53 | 22 | 33.2±15.9 |
| No. days oo lived | 6 | 10 | 6 | 11 | 6 | 6 | 30 | ? | 6 | 10.1±8.3 |

GROUP B (Single °)

| | Replicate No. | | | | | | | | | Mean/ replicate |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | |
| No. eggs/female | 213 | 0 | 18 | 1 | 93 | 82 | 159 | 43 | 343 | 105.8±108 |
| Mean eggs/°/day | 2.9±3.2 | 0 | 0.4±0.9 | 0.02±0.1 | 1.7±2.6 | 1.2±1.4 | 1.8±2.5 | 0.8±1.7 | 3.8±3.3 | 1.4±1.3 |
| Max No. eggs °/day | 12 | 0 | 3 | 1 | 11 | 7 | 10 | 7 | 11 | - |
| No. days °° laid eggs | 60 | 0 | 18 | 1 | 28 | 35 | 49 | 17 | 73 | 16.5±18.1 |
| No. days °° lived | 73 | 0 | 47 | 45 | 55 | 66 | 90 | 53 | 90 | 58.3±25.9 |

Among the females caged singly, one lived 90 days, average longevity was 58.3±25.9 days (N =9) in the cages. In this group one female laid 364 eggs. Maximum number of eggs laid by one female was 12. A total of 609 and 871 eggs were laid by group A and B respectively, and the males in group A lived 6-30 days, average 10 days (N =8). In group A 50% of the eggs hatched, and in Group B there was 80% hatch. The results of the experiment show that the presence of males in a confined area and frequent copulation lowers the oviposition rate and fertility. The peak oviposition period was late June and early July. Most females layed eggs until a few days before their death.

b) Pterolonche inspersa:

A field trial was started in 1985 for mass production of P. inspersa larvae, for shipment to California.

In May 1985, ca. 1300 C. diffusa rosettes were collected around Thermi and transplanted into a plot at the University Farm Thessaloniki. C. diffusa rosettes infested with mature Pterolonche larvae were collected in July 1985, in Thermi, and planted among the 1,300 C. diffusa rosettes (See Annual Report 1985) so the emerging adults had the chance to oviposit on these transplanted rosettes. The rate of infestation was not as high as expected.

In March 1986 the plot was weeded to save the C. diffusa plants, in our plot which were being crowded out by weeds. The weeds killed most of the C. diffusa plants, in the subsequent months. In July 1986, 15 of the original C. diffusa roots were dissected. Two roots contained 1 pupa each and 2 roots had one silken feeding tube each. The rest of the roots in the sample were not infested. About 50 other C. diffusa plants were found in the plot, but these were not examined. However, it was not a productive method for producing large numbers of P. inspersa larvae. From the lack of infestation, it seems that the adults left the plot even though no wild C. diffusa plants

were growing within a several kilometer radius from our plot. We concluded this is not a valid method for producing large numbers of insects. Perhaps managing adults in a cage or potted plants would prove to be more productive.

Some plants of C. diffusa (U.S. biotypes) were grown at the University Farm in 1985, near the local C. diffusa plants. There were 32 rosettes grown from Oregon seed and 32 rosettes from Washington seed. In July 1986, only 4 of the Oregon plants were found in the plot, and seven larvae (4,2,1,0/plant) were found in their roots. Ten of the Washington plants were found and dissected in July 1986 and five larvae (1,1, 1,2/plant) and 2 pupae (one/plant) were found in them.

Centaurea solstitialis Project

a) Larinus curtus:

This weevil is a possible candidate for biological control of YST because it oviposits in flowers and its larvae destroy the mature seeds. It is not common in all the areas searched in Greece so far, so an attempt was made to rear enough adults for host specificity studies in 1987.

For this rearing attempt, eight adults were collected in Doirani on June 20 and caged on YST branches at the University Farm on July 4. Later, 20 adults were collected in Thermi and caged on a large YST plant on July 8. All the seedheads (1600) on the caged plants were collected on September 3, and 100 of them were dissected.

When the YST seedheads were opened, it was evident that they had already been infested before they were caged with the beetles. Most of the seed feeders oviposit in unopened flower buds, while L. curtus oviposits in the opened flowers, therefore, the other species are already larvae of some age when the L. curtus eggs are deposited. Dissection of 100 dried flowerheads

produced only 3 living L. curtus and showed that 54% of the flowerheads were infested with various species (Trypetidae (several spp.), Isocolus and Lasioderma). It was also noted that Acanthophilus helianthi, which infested 17% of the flowerheads dissected was a very good competitor because in one seedhead the head capsule of the curculionid larva and the pupal exuvium of an A. helianthi were found. Parasites also were present in the cages, because 8 dead larvae and 3 parasitized C. curtus larvae were found in the seedheads dissected. The experiment shows that in order to rear L. curtus in a cage the YST plants must be caged before they produce buds.

C - Exploration: A trip was made to the islands of Rhodes and Kos, in April, searching for rosette feeders.

RHODES ISLAND

YST rosettes were examined in 9 locations in Rhodes. In each location 20-90 rosette roots (N =310) were dissected and the aerial parts of about 1500 rosettes (70-300/location) were examined for rosette feeders. Beside 2 species of leaf miners nothing interesting could be found. A small beetle was reared from a sample taken for the rearing of the leaf miners. The beetle has not yet been identified.

Kos Island

YST is much more common in Kos than in Rhodes. About 3,000 rosettes were examined at 8 locations and about 300 rosette roots were dissected (20-50/location) (from 0.5-1.5 hours were spent at the locations). A few rosettes infested with a rust, (probably Puccinia sp.) were found in 3 locations. Samples of infected leaves were sent to Dr. Wm. Bruckart in Frederick, MD. Besides the 2 species of leaf miners (same as in Rhodes) nothing else was found.

On both islands about 10% of the YST plants were bolting by mid-April. Carduus pycnocephalus was heavily attacked by a dipterous leaf miner, which caused considerable damage to the plants. About 50 pupae were reared from the sample but no adults were obtained, even though some of the pupae were kept in a refrigerator for two weeks (4°C) in hopes of breaking their hibernation. We have kept the pupae at Thermi, hoping to get adults in 1987. These same leaf miners are found at Thermi on C. pycnocephalus, but are much less common.

## SHIPMENTS MADE IN 1986

| Date | No. Shipped | Species Shipped | Destination |
|---|---|---|---|
| March 10 | 20 | *Euphorbia siguieriana* | Rome |
| " " | 50 | *Centaurea diffusa* pl. | " |
| March 17 | 20 | *Centaurea diffusa* pl. | " |
| April 2 | 100,000 | YST seedheads | Albany |
| April 9 | 700 | *Simyra* eggs | Rome |
| April 23 | 400 | *Simyra* eggs + 200 $L_1$ larvae | Rome |
| April 23 | 4 | Rust samples YST from Kos | Maryland |
| May 12 | 50 | *Euphorbia siguieriana* plants | Rome |
| " 27 | 700 | *Bangasternus orientalis* | Albany |
| " 28 | | *Centaurea diffusa* roots | Rome |
| " " | | *Bangasternus fausti* adults | Rome |
| June 3 | 625 | *Bangasternus orientalis* | Albany |
| " " | 50 | *Bangasternus fausti* (ex diffusa) | "1/ |
| " " | 50 | *Bangasternus orientalis* (ex *calcitrapa*) | "1/ |
| " 5 | 700 | *Centaurea cyanus* heads | Delémont |
| " 10 | 120 | *Centaurea diffusa* rosettes | Rome |
| " " | 230 | *Bangasternus orientalis* | Albany |
| " " | 4,000 | *Centaurea cyanus* heads | " |
| " " | | *Pterolonche* larvae in artificial diet | Albany |

1/ for isozyme studies by MADDOX at ALBANY, CA.

| Date | No. Shipped | Species Shipped | Destination |
|---|---|---|---|
| June 10 | 26 | Assorted thistle head samples (Turner project) | Albany |
| " 25 | 215 | Eustenopus adults | Rome |
| July 26 | 86 | assorted thistle head samples, 6 boxes(Turner project) | Albany |
| " " | | Convolvulus arvensis infested with Aceria convolvuli | " |
| " 16 | 5000 | Centaurea cyanus seed heads | Albany |
| " " | 65 | herbarium samples (Turner project) | Albany |
| Aug. 21 | 69 | Thistlehead samples, 3 boxes (Turner project) | Albany |
| Sept. 4 | | 153 Thistlehead samples, 7 boxes (Turner project) | Albany |
| Oct. 2 | | 40 Thistlehead samples, 2 boxes (Turner project) | Albany |
| Oct. 6 | | Pheromone traps + parasites, 1 box | Paris |

# INSECTS FOR IDENTIFICATION

| NAME OR KIND | ORIGIN |
|---|---|
| *Urophora sirunaseva* | reared from YST from Rhodes |
| *Terellia virens* | reared from YST from Rhodes |
| *Urophora sirunaseva* | reared from YST from Kos |
| Trypetid flies | reared from *Centaurea cyamus* |
| *Chaetorellia* | collected on *Nothobasis syriacus* |
| Trypetid flies | reared from *Carthamus dentatus* |
| *Terellia* | collected on YST in Doirani |
| *Chaetorellia* | reared from *C. cyanus* |
| small beetles (leaf miners) | reared from YST |

Visitors:

G. Campobasso: May 15 - 26

P. Dunn : June 19 -21

D. Perkins : August 18 and 25

# PAPERS PUBLISHED AND MEETINGS ATTENDED

D. M. Maddox and R. Sobhian, A Field Experiment to Determine Dispersal, Attraction and Oviposition behavior of Bangastermus orientalis and B. fausti (Col: curc.), Biological Control Candidates for Yellow Starthistle and Diffuse Knapweed Env. Ent., in press.

D. M. Maddox, R. Sobhian, b. Joley, A. Mayfield and S. Supkoff New biological control for yellow Starthistle Calif. Agric., Nov. - Dec. 1986

L. Fornasari attended "The First International Congress of Dipterology", Budapest. August 17-24, 1986

P. H. Dunn attended the Internation Symposium on Germplasm Addis Ababa. October 12-17.

P. H. Dunn attended European Laboratories meeting, October 19-22, with Kincaid and Army

P. Pecora attended the Annual European Working Group Meeting on Biological Control of Weeds, Copenhagen. November 3-7

P.H. Dunn attend the Entomological Society of America National Meeting, Reno, Nevada. November 24-28.

TRAVEL (Rome, Laboratory)

| | |
|---|---|
| February 24-March 1 | Sobhian to Rome en route to Athens and Thessaloniki from Vienna. |
| February 27-March 1 | Clement to Florence |
| March 5-6 | Fornasari, Stazi to Pisa, leafy spurge field work |
| March 17-20 | Mimmocchi, Cristofaro to Milano, Computer specialization |
| March 26-27 | Fornasari, Stazi to Pisa, leafy spurge field work |
| April 11-21 | Sobhian to Athens, Pireaus, Rhodes, Kos, |
| April 14-15 | Fornasari, Stazi to Pisa and Monteverdi, leafy spurge field work |
| April 28-May 4 | Campobasso to Bari, Centaurea field work |
| May 7-8 | Pecora, Fornasari to Pisa, leafy spurge field work |
| May 17-25 | Sobhian to Drama |
| May 26-27 | Pecora, Cristofaro to Pisa, leafy spurge field work |
| May 31-June 8 | Sobhian to Ptolemaida, |
| June 4-5 | Pecora to Pisa, leafy spurge field work |
| June 6-21 | Dunn, Zagreb, Belgrade, Thessaloniki, confer with Plant Protection Institute in Zagreb and administrative staff of the American Consulate in Thessaloniki, and survey and field collection of Bangasternus and Eustenopus with Sobhian |
| June 15-24 | Pecora, Fornasari to Romania and Austria, leafy spurge field work |
| June 11-25 | Sobhian with Dunn to Argos, Mili, Kiato, Halkida, Pirgadikia, Delfi, Lamia, Eptahori |
| June 10-12 | Cristofaro, Stazi to Pisa, leafy spurge field work |
| June 14-22 | Campobasso to Bari, Centaurea field work |
| June 18-19 | Cristofaro, Stazi to Pisa, leafy spurge field work |
| June 30-July 1 | Cristofaro, STazi to L'Aquila, leafy spurge field work |

| | |
|---|---|
| July 3-4 | Cristofaro, Stazi to Pisa, leafy spurge field work |
| July 11-15 | Pecora to Vienna, leafy spurge field work |
| July 31-August 5 | Sobhian Peloponesus, Lamia |
| July 29-August 7 | Vincenti to Beltsville - administration |
| August 11-15 | Sobhian to Lamia, Agrinio, delfi, Aliartos |
| August 26-30 | Sobhian Neopoli, Eptahori, Malakasi, Olympos |
| September 18-22 | Sobhian to Githio, Kalamata, Stimfalia |
| September 18-23 | Pecora to Hungary, leafy spurge field work |
| September 22-23 | Cristofaro to Pisa, leafy spurge field work |
| September 24-25 | Sobhian to Komotini |
| October 22-23 | Fornasari, Cristofaro to Pisa, leafy spurge field work |
| October 19-25 | Pecora, Stazi to Romania, leafy spurge field work |
| November 28-Dec. 19 | Dunn to U.S. consultation |
| December 10-11 | Stazi to Pisa, leafy spurge field work |

# INSECT AND PATHOGEN SHIPMENTS

## ROME LABORATORY

| ORGANISM | TARGET WEED | NUMBER AND STAGE | DATE | DESTINATION |
|---|---|---|---|---|
| *Urophora cardui* | Canada thistle | 732 galls | 4/1/86 | Albany, CA. |
| *Bayeria capitigena* | Leafy spurge | 600 larvae | 4/1/86 | Albany, CA. |
| *Dasineura capsulae* | Leafy spurge | 1,870 larvae | 4/1/86 | Albany, CA. |
| *Dasineura capsulae* | Leafy spurge | 840 larvae | 4/1/86 | Albany, CA. |
| *Dasineura capsulae* | Leafy spurge | 670 larvae | 4/1/86 | Albany, CA. |
| *Bayeria capitigena* | Leafy spurge | 170 galls with larvae and pupae | 5/7/86 | Albany, CA. |
| *Bayeria capitigena* | Leafy spurge | 600 larvae | 5/28/86 | Albany, CA. |
| *Oberea erythrocephala* | Leafy spurge | 90 adults | 5/28/86 | Albany, CA. |
| *Bayeria capitigena* | Leafy spurge | 310 galls | 6/5/86 | Albany, CA. |
| *Oberea erythrocephala* | Leafy spurge | 228 adults | 6/5/86 | Albany, CA. |
| *Aphthona flava* | Leafy spurge | 178 adults | 6/5/86 | Albany, CA. |
| *Bayeria capitigena* | Leafy spurge | 230 galls | 6/13/86 | Albany, CA. |
| *Oberea erythrocephala* | Leafy spurge | 200 adults | 6/13/86 | Albany, CA. |
| *Aphthona flava* | Leafy spurge | 1,100 adults | 6/17/86 | Albany, CA. |
| *Aphthona cyparissiae* | Leafy spurge | 350 adults | 6/27/86 | Albany, CA. |
| *Aphthona czwalinae* | Leafy spurge | 350 adults | 6/27/86 | Albany, CA. |
| *Bayeria capitigena* | Leafy spurge | 300 galls with larvae and pupae | 7/4/86 | Albany, CA. |

| ORGANISM | TARGET WEED | NUMBER AND STAGE | DATE | DESTINATION |
|---|---|---|---|---|
| Aphthona flava | Leafy spurge | 950 adults | 7/4/86 | Albany, CA. |
| Uromyces scutellatus | Leafy spurge | spores | 7/7/86 | Ft. Dietrich, Md. |
| Centaurea alba | Diffuse knapweed | 2,000 flower heads | 7/15/86 | Crowe & Co. AG, Basel, Switzerland |
| Aphthona czwalinae | Leafy spurge | 200 adults | 7/15/86 | Albany, CA. |
| Aphthona cyparissiae | Leafy spurge | 800 galls | 7/15/86 | Albany, CA. |
| Centaurea solstitialis | YST | numerous | 7/15/86 | I. Pittara, Thessaloniki, Greece |
| Centaurea solstitialis | YST | numerous | 7/29/86 | I. Pittara, Thessaloniki, Greece |
| Centaurea solstitialis | YST | numerous | 8/12/86 | I. Pittara, Thessaloniki, Greece |
| Chondrilla juncea L. | YST | 50 seed samples | 8/14/86 | J. Cullen, CSIRO, Montpellier, France |
| Centaurea sphaerocephala | YST | 1,000 capitula ca. | 8/26/86 | D. Schroeder, CIBC, Delémont, Switzerland |
| Dasineura capsulae | Leafy spurge | 2,000 larvae | 11/18/86 | Albany, CA. |

VISITORS

(in order of visit)

ROME LABORATORY

Dr. J-P. Aeschlimann, CSIRO, France

Dr. T.J. Army, ARS-USDA, NPS Leader, Beltsville Md.

Dr. Bruce Auld, Orange, Australia

Mrs. Nolie Bentley, Washington D.C.

Dr. Maurizio Biondi, Ministero dell'Agricoltura e delle Foreste

Dr. Marco Bologna, Unversity of L'Aquila

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Dr. Jim Cullen, CSIRO, France

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Dr. Kenneth Hagen, Division of Biological Control, University of California, Berkeley, CA.

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Mr. D. R. Kincaid, USDA-ARS, Director International Activities, Beltsville, Md.

Mrs. Margo Kincaid, USDA-ARS, Secretary to Dr. Army, Beltsville, Md.

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Dr. Ian White, C.I.E., London, United Kingdom

Partial List of Recipients of this Report

Agricultural Counselor, American Embassy, Rome, Italy

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Asian Parasite Lab., Seoul, Korea

CIBC, Trinidad, West Indies

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